Peer Review File

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Reviewer A

Your manuscript deserves publication after a minor revision.

I have only two comments:
1) Lines 75-83 You cite works where the PEG covered AuNP were used of different diameters as yours. Please clarify this fact, since the coverage and the size, as well as the concentration of NP, could make difference.

Answer) We are so sorry for error. We revised it.
Gold nanoparticles, about 5 nm in diameter, were purchased from Cytodiagnostics (Burlington, ON, Canada).

2) You demonstrated important results of the important radiosensitization effects for AuNP. However, as it is clear that interpretation of the results is difficult, what I propose to you is to consider this effect:
as discussed in the work https://doi.org/10.3390/nano11030727 Selective Oxidation of Transient Organic Radicals in the Presence of Gold Nanoparticles.
Shcherbakov et al wrote in their paper:
"Our results have significant consequences on the understanding of the mechanism of AuNPs as radiosensitization. Nowadays, it is considered to occur through AuNPs' effect on different physical, chemical, and biological processes [24–28]. The radiosensitization mechanism is often discussed in the context of ROS overproduction in the presence of AuNPs [25,27]. However, AuNPs' catalytic properties towards radicals were never discussed before. It is essential to mention that different organic radicals are always present in cells due to their metabolism. Therefore, AuNPs can change the cell chemistry affecting radicals' processes, even BEFORE IONIZING radiation is applied, which, in principle, could shatter the cell's RESISTIVITY towards ionization radiation"

Thus it would be interesting based on your results to consider the catalytic effect of AuNP for tumour cells and healthy ones. It could mean that by catalyzing some cellular REDOX reactions in the cancerous cells leads to their low resistivity to ionization.

In the presence of oxygen AuNP "Characterization of glucose oxidation by gold nanoparticles using nanoceria" doi.org/10.1016/j.jcis.2014.04.025 can oxidize even glucose, thus many molecules within the cell could be oxidized VitC, Glutothion, etc.

In this way, maybe you can discuss in your work these subjects.

Answer) As your request, we have added the description and references in the revised manuscript.

Reviewer B

The results show almost no effects of gold nanoparticles on dose delivered to the normal cells which is not what most researchers have found for instance Rahman-09 and many others. If the authors would like to keep these results, they have to include strong scientific argument to accept that.
Why did you use MTT for the evaluation of cells viability? Such a technique will not show the cells which will die later.
The other comments are in the attached copy of the manuscript please adhere to those all.
Answer) We need to check it with in vivo but these events can be explained based on these references. Thus we added these descriptions and references in the revised manuscript.

Ref1) Selective Oxidation of Transient Organic Radicals in the Presence of Gold Nanoparticles.:
"Our results have significant consequences on the understanding of the mechanism of AuNPs as radiosensitization. Nowadays, it is considered to occur through AuNPs' effect on different physical, chemical, and biological processes. The radiosensitization mechanism is often discussed in the context of ROS overproduction in the presence of AuNPs. However, AuNPs' catalytic properties towards radicals were never discussed before. Notably, cells are always found to comprise various organic radicals because of the way their metabolism works. It is for this reason that AuNPs are capable of altering the cell chemistry that affects the processes of radical, even prior to the application of ionizing radiation, which can principally obliterate the resistivity of the cell toward radiation ionization
Therefore, our findings were taken into consideration for AuNP's catalytic impact for healthy and tumor-infested cells. This implies that catalyzing certain REDOX reactions within the cancerous cells results in their low ionization resistivity.

Ref2) In the presence of oxygen AuNP
"Characterization of glucose oxidation by gold nanoparticles using nanoceria" doi.org/10.1016/j.jcis.2014.04.025 can oxidize even glucose, thus many molecules within the cell could be oxidized VitC, Glutothion, etc.

Cell death was checked for apoptosis through FACS analysis and western blotting after treatment with AuNPs and radiation, and MTT assay was performed to confirm cell viability.

Reviewer C

Overall, I found the manuscript to be well written, technically solid, and interesting. Although the data is convincing, the novelty of the study is limited.
In other words, this paper provides only an incremental advance in our standing of toxicity of AuNPs on normal Hepatocytes.
The authors need to perform additional experiments to substantiate different AuNP sizes.
Addressing this concerns would strengthen the conclusion of the manuscript.
Answer) We are currently performing experiments for normal damage in regard to different AuNP sizes and preparing a separate paper about the detailed experimental results. We hope for the reviewer's and editor's understanding about this.

Reviewer D

There are many articles published on the use of gold nanoparticles to treat cancers. However, I don't see much work done on the toxicity of gold nanoparticles on normal cells, especially in the presence of radiotherapy. Thus, I believe this work is original and publishable. The authors need to address the following concerns.

1. As the authors mentioned that normal hepatocytes are sensitive to X-ray radiation, my first question is whether the X-ray radiation used in this work is high?
Answer) The range of dose at 1-5Gy is included for low dose.

2. Authors are suggested to add a space between numbers and units.
Answer) We revised them.

3. Authors need to add some experimental details on how you coat PEG on the AuNPs.
Answer) We are so sorry for error. We revised it.
Revised point:
Gold nanoparticles, about 5 nm in diameter, were purchased from Cytodiagnostics (Burlington, ON, Canada).

4. Authors have to rewrite the following sentences.
"we found that the effect of AuNPs and IR neither additive nor synergistic..." and "These results suggest that AuNPs may not be able to radiosensitize normal hepatocytes through the enhancement of irradiation-induced apoptosis." For the second one, I guess the authors were trying to say "in order to" instead of "through ..."
Answer) As your request, we revised them in the revised manuscript.

According to Valeriote and Carpentier with formula, we found that the effect of AuNPs and IR neither additive nor synergistic on normal hepatocytes .
These results suggest that AuNPs may not be able to radiosensitize normal hepatocytes for the enhancement of irradiation-induced apoptosis.

5. In vitro and in vivo have to be written in italic.
Answer) As your request, we revised them.

6. References are a bit old.
Answer) As your request, we revised and added new one.

7. Figure captions are a bit unclear, especially Fig.1. Authors are suggested to be consistent with the way to name AuNPs.
Answer) As your request, we revised them.