We would like to thank the editor and referees for their feedback and valuable comments on the manuscript. Our point-by-point responses are presented below (in blue color), with new or modified text in italics, whereas the manuscript has the new text highlighted in yellow.

Revisor 1

Major points

R1.1 In INTRODUCTION, the most relevant studies are not discussed, for example,

[1] Dolan D W, Zupanic A, Nelson G, Hall P, Miwa S, Kirkwood T B and Shanley D P, 2015. Integrated Stochastic Model of DNA Damage Repair by Non-homologous End Joining and p53/p21-Mediated Early Senescence Signalling. PLoS Comput Biol, 11, e1004246.

[2] Hu, Ankang et al."Modeling of DNA Damage Repair and Cell Response in Relation to p53 System Exposed to Ionizing Radiation." International journal of molecular sciences vol.23,19 11323. 26 Sep.2022, doi:10.3390/ijms231911323.

[3] Hat B, Kochanczyk M, Bogdal M N and Lipniacki T, 2016. Feedbacks, Bifurcations, and Cell Fate Decision-Making in the p53 System. PLoS Comput Biol, 12, e1004787.

We acknowledge the reviewer for providing those references. We extended the introduction accordingly on lines 109-110, from:

"Kinetic models for this protein reaction network coupled with DNA repair models have been published elsewhere [Ma L., et al (2005), Zhang X. P. et al (2009), Zhang X. P., et al (2011)]."

To:

"Kinetic models for this protein reaction network coupled with DNA repair models have been published elsewhere [Ma L., et al (2005), Zhang X. P. et al (2009), Zhang X. P., et al (2011)]. For Monte Carlo modeling, Hu et al. [Hu A., et al (2022)] presented a multiscale platform to simulate the radiation and cell response in a single-cell approach, that integrates NASIC (Nanodosimetry Simulation Code) for calculating the initial distribution of DNA lesions, followed by DNA damage repair model based on an NHEJ pathway [Dolan D., et al. (2015)], and a model for the p53 protein network [Hat B. et al (2015)] including the influence on the cell cycle regulation."

R1.2. In 2.2.3. DSB assignment, the estimation of DSB is slightly rough and only applies to low LET, the expectation of DSB number depends only on the nuclear dose, the literature-based [59] is old, is it possible to obtain more advanced methods (e.g., DNA-model-based track structure simulation) and apply on more cases?

Indeed, our work was focused on low LET X-ray irradiation. For this specific LET range, where the current DSB assignment approach holds acceptable validity, we used the Poisson approach assignment to speed-up the testing of the coupling with the p53 network model. Nevertheless, the proposed framework can accommodate much more advanced methods through the TOPAS-nBio capabilities. We clarified in the text as follows:

We changed in section *2.2.3. DSB assignment* (lines 221-223) from:

"In a cell population irradiated with a uniform particle field at low LET (~0.2 keV/μm) radiation, the number of DSBs can be computed from a Poisson distribution, and the mean number of lesions increases linearly with the absorbed dose [Rothkamm K. & Löbrich M. (2003)]"

To:

"In a cell population irradiated with a uniform particle field at low LET (~0.2 keV/μm) radiation, a reasonable approximation to compute the number of DSBs is from a Poisson distribution, where the mean number of lesions increases linearly with the absorbed dose [Rothkamm K. & Löbrich M. (2003)]. This approach was adopted in the current work to facilitate the coupling and speed-up the testing of the p53 network model. Nevertheless, TOPAS-Tissue is not limited to the assignment of initial DNA damage using the Poisson distribution approach. More advanced methods to compute DSB are available in TOPAS-nBio, and can be accessed through the proposed framework. For example, the DBSCAN algorithm, and a whole DNA cellular nucleus model are available in the TOPAS-nBio suite of scorers among several others [Schuemann J., et al (2019)]."

R1.3. To support the significance of the study, RESULT needs more information and verification about p53 and the DNA repair module, namely, discussions on how p53 and DNA repair affect the result of simulation are suggested be supplemented.

We agree, due to limits in the manuscript length, we provide such information for the DNA repair and p53 protein network in a supplementary document.

R1.4. The cell culture growth in this paper seems to be mainly aimed at in-vitro culture, there is still a certain gap with human tissues. We expect the TOPAS-Tissue framework to be further mature, for example, considering in-vivo tissue environment and cell interactions, so as to truly expand to the tissue level effect in the future.

Thanks for pointing this out. TOPAS and CC3D are capable of handling more complex biological models. However, we selected an in-vitro culture as it provides a simple and accurate mean to validate the framework experimentally. The simulation of tissue has been shown in CC3D in [Shirinifard A., et al (2009)]; and we presented an application of TOPAS-tissue (under review) for studying irradiation of vascularized tumors with mini-beam radiotherapy.

We added a paragraph to the end of the section *2.2.2. Irradiation stage*:

"Due to the CC3D intrinsic nature of implementing geometrical models in a grid geometry and the capabilities of TOPAS/TOPAS-nBio to generate voxelized geometries, TOPAS-Tissue offers a flexible platform to irradiate any CC3D geometrical model. Other types of cells, cell population arrangement (tissue), and their behavior can be incorporated by defining new phenotypes in CC3D. The parameters to define such phenotypes might include, the growth rate, volume, topological shape, among many others; ongoing developments to incorporate PhenoCellPy [Gianlupi J. F., et al (2023)] shall be presented in future works."

Revisor 2

R2.1. Indicate the novelty of this work compared to existing literature in abstract

We added the following lines

"TOPAS-tissue extends previous works by simulating in an end-to-end way the effects of radiation in a cell population, from irradiation and DNA damage leading to the cell fate."

R2.2. The introduction section lacks sufficient background information and requires significant improvements. It should include a recent literature review on the biological effects of radiation for new readers.

We added a few lines in the introduction (lines 40-46) to reflect that this work focuses on effects of ionizing radiation, and the main focus on the biological effect after interaction of radiation with cells. Changed from:

"These codes have been developed to simulate DNA damage under different irradiation conditions and to explore the spatial structure of DNA strand breaks and DNA repair. Previous works reported the spatial distribution of DNA single-strand breaks (SSB) and double-strand breaks (DSB) induced by direct and indirect actions of radiation in geometrical models of DNA, ranging from DNA plasmids, chromatin fibers, and whole cell nucleus, see (and references therein) [Friedland W., et al (2003), Incerti S., et al (2010), Nikjoo H., et al (1997), Dos Santos M., et al (2014), Bernal, M. A., et al (2015), Tran H. N., et al (2021), D-Kondo, N., et al (2021), Poignant F., et al (2022), Ramos-Méndez J., (2022)]"

To:

"These codes have been developed to simulate DNA damage under different irradiation conditions and to explore the spatial structure of DNA strand breaks and DNA repair. The main target of ionizing radiation in biological tissue is the nuclear DNA. It can produce a variety of lesions, including DNA Double Strand Breaks (DSB), defined as two DNA Strand Breaks (SSB) produced on opposite strands of the DNA separated by less than 10 base pairs [Nikjoo H., et al (2016)]. DSB are difficult to repair by the cell. When DSBs accumulate unrepaired or mis-repaired, they can lead to gene mutations and chromosomal aberrations which eventually lead to cellular death [Pfeiffer P., et al (2000), Foster J., et al (2019)].

The breaks can be produced directly when ionizing radiation deposits energy on the DNA molecule, producing physical damage [M.DosSantos et al (2014)]; or indirectly, when free radicals are produced in the medium, that can react with the DNA components and produce chemical damage [Tomita H. et al, (1997), Mumtaz S., et al (2022)]. The initial distribution of lesions depends on the Lineal Energy Transfer (LET) of the radiation used, which is a parameter that characterizes the density of ionizations on the medium that the radiation is traversing [Charlton, D. E., et al (1997), Nikjoo H., et al (2016)].

Previous works reported the spatial distribution of DNA breaks induced by direct and indirect actions of radiation in geometrical models of DNA, ranging from DNA plasmids, chromatin fibers, and whole cell nucleus, see (and references therein) [Friedland W., et al (2003), Incerti S., et al (2010), Nikjoo H., et al (1997), Dos Santos M., et al (2014), Bernal, M. A., et al (2015),

Tran H. N., et al (2021), D-Kondo, N., et al (2021), Poignant F., et al (2022), Ramos-Méndez J., (2022)]"

R2.3. Add information regarding the interaction of radiations with the biological systems for new readers. Incorporation of the following recent article in the introduction section might be helpful [https://doi.org/10.3390/ijms23169288].

We included the reference. Please see also the response R2.2.

R2.4. How does TOPAS-Tissue perform with other cell types or 3-D cell cultures beyond the PC-3 model?

Please see response R1.4

R2.6.H Provide a more detailed explanation of the parameter selection process for the DNA repair and cellular response models, particularly the p53 pulse model and the two-lesion kinetic model?

We agree. Due to the limit in the manuscript length, we provide such information in a supplementary document. In addition, we refer to such document (line 272-273):

"The coupled model was validated elsewhere for MCF7 human breast cancer cell assays treated with the radiomimetic drug doxorubicin. It was reported that a maximum of 5 pulses was observed for apoptotic cells over a period of 6hr between oscillations."

To:

"The coupled model was validated elsewhere for MCF7 human breast cancer cell assays treated with the radiomimetic drug doxorubicin. The parameters, reported in Zhang, X. P. et al (2011), are listed in the supplementary material. To verify the implementation of the model, the time evolution curves of the concentration from p53, ATM* and Wip1 pulses were compared with those from Zhang et al., (2011). Experimentally it was reported that a maximum of 5 pulses was observed for apoptotic cells over a period of 6hr between oscillations."

R2.7. What are the limitations and assumptions inherent in the current DNA repair and cell response models, and how do these assumptions impact the simulation results?

Thank you for pointing that out, we agree that the description of limitations and assumptions for those modes are needed. To address these points, we added the following paragraphs at the discussion section (after line 479):

"The TLK model used in this work considers two repair pathways corresponding to slow and fast kinetics. This is a simplification of the actual repair pathways, as it does not consider explicitly the reactions of involved proteins. Therefore, cells with deficient protein expressions like the XPF-deficient human fibroblasts (XFE) cannot be simulated with this model. Models that consider an extended set of protein reactions have been reported elsewhere [Belov O. et al (2015)]. However, the pathway for cell fate in the previous work is not considered.

In this work, the cell fate is simulated with a p53 protein network model. Nevertheless, other cell behaviors induced by radiation like cell cycle regulation are not considered. A model describing the effect on cell cycle regulation has been reported elsewhere [Iwamoto K., et al (2011)]. This and other models to simulate distinct cell behaviors can be added to TOPAS-tissue in SBML format through CC3D".

R2.8. How does the framework handle different types of ionizing radiation, such as protons or heavy ions, which have different energy deposition patterns? Discuss in the manuscript.

TOPAS-tissue relies on TOPAS and TOPAS-nBio tools which are multiple-particle Monte Carlo tools. Thus, other types of radiation can be simulated. We added further information in lines 357-359 by changing from:

"Future implementations of scoring methods will consider the TOPAS-nBio Monte Carlo track-structure to assign SSB and DSB required by the DNA model repair (section 2.2.3.)."

To:

"TOPAS-tissue is compatible with the TOPAS-nBio Monte Carlo track-structure. In this way, the SSB and DSB required by the DNA model repair (section 2.2.3.) can be simulated with TOPAS-nBio for a large set of particles and energies, including microdosimetric and nanodosimetric quantities [Faddegon B., et al (2023)]."

R2.9. How does the framework integrate external factors influencing cell behavior, such as oxygen availability, nutrient gradients, and mechanical stress, which can affect radiosensitivity?

Those factors can be included in the CC3D configuration to evaluate radiosensitivity after irradiation with TOPAS/TOPAS-nBio. We added a clarification on the discussion (after line 464) section as follows:

"There are several factors that influence the cell radiosensitivity such as oxygen availability, nutrient gradients, and mechanical stress. CC3D has tools to handle all these factors as diffusive chemical fields. In this way, oxygen and nutrients distribution and cellular intake-uptake can be accounted for [Shirinifard A., et al (2009)]. Mechanical stress is simulated with the Potts algorithm of CC3D by e.g., tracking automatically quantities like the internal pressure of each cell that is directly related to the mechanical stress [Swat M. H., et al. (2012)]"

R2.10. The paper contained typos and grammatical errors. Double-check and correct them in the revised version.

Thanks for pointing this out, we have revised the document and corrected typos.