Investigation of the bismuth oxide nanoparticles on bystander effect in MCF-7 and hFOB 1.19 cells

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Abstract. This study aims to investigate the effect of bismuth oxide nanoparticles (Bi2O3 NPs) on the radiation induced bystander effect (RIBE) in MCF-7 and hFOB 1.19 cells line. The cells were irradiated with radiation doses of 0 to 12 Gy using 6 MV photon beam in a single exposure. The irradiated cells’ culture media were transferred to non-irradiated bystander cells 1 hour post-irradiation. PrestoBlue assay was then performed in this experiment to assess the cells’ viability. Results of cell viability percentage in all bystander cell groups compared to the control showed no significant differences (P > 0.05) for both MCF-7 and hFOB 1.19 cell lines. The test also revealed no radiation dose dependencies for all bystander cells groups. The present study demonstrated that MCF-7 and hFOB 1.19 bystander cells were able to proliferate (> 80%) after 48 hours incubation with irradiated-cell conditioned medium (ICCM) treated with Bi2O3 NPs. In summary, the use of Bi2O3 NPs for radiosensitization in radiotherapy is safe and do not increase the RIBE responses in non-targeted cells. RIBE remained as one of the most crucial factors that need to be address and considered for the application of nanoparticles as radiosensitizers in radiotherapy.

1. Introduction
In an effort to improve the efficacy of radiotherapy, nanoparticles were introduced in a way that the radiotoxicity in the cancerous region of human tissues can be increased. The unique properties of NPs such as high surface area, stable and tunable, make them ideal for the transportation of chemotherapeutic and phototherapeutic agents, as well as application as radiosensitizers [1]. Extensive investigations have been conducted on the radiation enhancement effect by gold nanoparticles (AuNPs) and other types of nanoparticles such as bismuth NPs (BiNPs). Both AuNPs and BiNPs have been reported as an attractive radiosensitizers that can increase the effect of radiation in tumors [2,3].

Over the years, attention in radiobiological studies has been widened to address the non-targeted effects of adjacent tissue surrounding the targeted area. Radiatian-induced bystander effects (RIBE) describe a situation where cells that have not been directly exposed to ionizing radiation behave as though they have been exposed. RIBE mainly expand the radiotoxicity effects to the non-targeted area.
The application of metal nanoparticles as radiosensitizer during the treatment rises the issue on whether they could enhance the RIBE towards the non-irradiated cells [4]. Despite some promising investigations on RIBE, the understanding of the mechanism and responses between the irradiated cells and adjacent healthy cells in the presence radiosensitizers during radiation treatment are still lacking. The present in-vitro study intended to investigate the effect of bismuth oxide nanoparticles (Bi$_2$O$_3$ NPs) on the bystander effects in human breast cancer cells (MCF-7) and human normal osteoblast cells (hFOB 1.19) irradiated with 6 MV photon beam.

2. Materials and Methods

2.1. Cell Culture and Bi$_2$O$_3$ incorporation
MCF-7 and hFOB 1.19 cell lines used in the experiments were grown in tissue culture flasks containing Dulbecco's modified Eagle's medium (DMEM) which is supplemented with 10% fetal bovine serum and 1% antibiotics (10,000 units/mL penicillin and 10,000 μg/mL streptomycin) (Nacalai Tesque, Kyoto, Japan). The cells were maintained in the incubator at 37 °C, 95% humidity, and 5% CO$_2$. Targeted cell lines were seeded at a density of $2 \times 10^5$ cells per flask 25 cm$^2$. Bi$_2$O$_3$ NPs were prepared by using a hydrothermal method, as reported in our previous study [5]. Before irradiation, 5 µMol/L of Bi$_2$O$_3$ was added into the targeted flask. Previous study reported that Bi$_2$O$_3$ NPs below 50 µg/mL concentration were not toxic to cells [6].

2.2. Irradiation and medium transfer
The targeted cells were prescribed to irradiation doses up to 12 Gy with 6 MV photon beam (Primus medical linear accelerator, Siemens Healthcare, USA), with a constant dose rate 300 cGy/minute in the beam field of 10 × 10 cm$^2$. After irradiation, the flasks were placed in an incubator at 37°C for 1 hour. The transfer of the medium was set up according to the technique developed by Mothersill and Seymour [7]. The irradiated cell-conditioned medium (ICCM) from the targeted flasks were then extracted and filtered through 0.22 μm polyethersulfone (PES) membrane filters to ensure that no cells were present in the transferred medium.

2.3 Cell Viability Assay
Cell viability experiments were performed using PrestoBlue cell viability reagent. The bystander cells were seeded at a density of $3 \times 10^3$ cells/well in 96-well plates. After 48 hours of incubation with ICCM, the samples were washed twice with phosphate-buffered saline (PBS). PrestoBlue reagent was added in each well followed by a 2 hours incubation. The fluorescence was measured using a microplate reader (Varioskan Flash, Thermo Scientific) at an excitation wavelength of 560 nm and an emission wavelength of 590 nm.

2.4 Statistical analysis
Data were expressed as mean ± standard error of the mean (SEM). One-way ANOVA was used to determine the significance of differences between bystander cells cultured in the sham-irradiated medium and irradiated medium. Differences were considered to be statistically significant when the $P$-values were less than 0.05.

3. Results and Discussion
Figure 1 shows the bystander responses in the non-irradiated cells measured after 48 hours incubation with the ICCM collected from the targeted cells. The range of viability for the control and bystander cells for MCF-7 and hFOB 1.19 cells were 93.9 – 100% and 91.5 – 100%, respectively. The percentage of cell viability in MCF-7 and hFOB 1.19 bystander cells groups compared to the controls showed no significant differences ($P > 0.05$). The cell viability results showed no radiation dose dependence for all bystander cells groups. RIBE responses of the bystander cell groups were not
statistically different from each different dose groups. This situation indicates that the RIBE response in MCF-7 and hFOB 1.19 bystander cells were independent of the prescribed radiation dosage.

Percentages of cell viability for the bystander cells incubated with ICCM from 2 Gy treated with Bi$_2$O$_3$ NPs were shown in Figure 2. We observed that the cell viability of MCF-7 and hFOB 1.19 bystander cells were 87.6 – 100% and 86.4 – 100% after receiving ICCM from irradiated cells treated with Bi$_2$O$_3$ NPs, respectively. The ICCM from targeted cells treated with Bi$_2$O$_3$ NPs decreases approximately 10% of the hFOB 1.19 bystander cells viability at 2 Gy. The increment in percentage of cell death suggests the presence of RIBE responses in hFOB 1.19 bystander cells groups. In the case of MCF-7 cells, no significant differences were observed between the bystander cells which received medium from target cells treated with Bi$_2$O$_3$ NPs and bystander cells which received medium from non-treated target cells ($P > 0.05$). Our data shows that the ICCM treated with Bi$_2$O$_3$ NPs did not increase cell death in MCF-7 bystander cells. The reduction in cell viability of MCF-7 cells is less than 5%. These results demonstrated that the bystander cells able to proliferate well after receiving conditioned medium from targeted cells treated with Bi$_2$O$_3$ NPs. This situation may indicate that Bi$_2$O$_3$ NPs are not toxic and do not significantly contribute to the increment of bystander responses in the non-targeted cells.

![Figure 1](image1.png)

**Figure 1.** Cell viability percentages for MCF-7 and hFOB 1.19 cells at different radiation doses

The present study indicates that the bystander effect in non-irradiated cells does not depend on the radiation dose received by the cell population. These results are in agreement with other reports of the dose independence of the bystander effect induced by conditioned medium harvested from irradiated cells [4,8]. Previous studies show that every cell within a population has the potential to release a bystander signal but however, the sensitivity of cells in responding to a bystander signal may depend on intrinsic cell characteristics [8].

![Figure 2](image2.png)

**Figure 2.** Cell viability percentage for MCF-7 and hFOB 1.19 cells incubated with ICCM from 2 Gy treated with Bi$_2$O$_3$ NPs
4. Conclusions
Our current data demonstrated that the irradiated Bi$_2$O$_3$ NPs particularly do not significantly increase RIBE responses in non-irradiated cells. This analysis is important in considering the current effort to safely deliver the NPs to enhance tumor cells death while minimizing the non-targeted effect on the normal tissue.

5. References
[1] Goel S, Ni D and Cai W 2017 ACS Nano 11 5233–7
[2] Abidin S Z, Zulkifli Z A, Razak K A, Zin H, Yunus M A and Rahman W N 2019 Mater. Today Proc. 16 1640–5
[3] Rahman W N, Bishara N, Ackerly T, He C F, Jackson P, Wong C, Davidson R and Geso M 2009 Nanomedicine Nanotechnology, Biol. Med. 5 136–42
[4] Rostami A, Toossi M T B, Sazgarnia A and Soleymanifard S 2016 Radiat. Environ. Biophys. 55 461–6
[5] Abidin S Z, Zulkifli Z A, Razak K A, Zin H, Yunus M A, Hasyimah N, Wail N, Rashid R A and Rahman N 2017 Mater. Today Proc. 00 0–6
[6] Ahamed M, Akhtar M J, Khan M A M, Alrokayan S A and Alhadlaq H A 2019 Chemosphere 216 823–31
[7] Mothersill C and Seymour C 1997 Int. J. Radiat. Biol. 71 421–7
[8] Shao C, Folkard M, Michael B D and Prise K M 2005 Int. J. Cancer 116 45–51

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