Buletin SK 9(1)(2025): 32 – 39
https://doi.org/10.17576/bsk-2025-9(1)-04 ISSN 2250-1852

Artikel Asli/*Original Article*

Influence of Bismuth Oxides Nanoparticles on Cellular Migration and Reactive Oxygen Species (ROS) Production in Photon Beam irradiated Cancer Cells

Kesan Nanopartikel Oksida Bismut terhadap Migrasi Sel dan Penghasilan Spesis Oksigen Reaktif (ROS) oleh Sel Kanser yang teradiasi Sinar Foton

RAIZULNASUHA AB RASHID[1], KHAIRUNISAK ABDUL RAZAK[2,3], SAFRI ZAINAL ABIDIN[4], HAFIZ M. ZIN[4], WAN NORDIANA RAHMAN[5,6*]

[1]Centre for Diagnostic Nuclear Imaging, Universiti Putra Malaysia, 43300 Seri Kembangan, Selangor.

[2]School of Materials and Mineral Resources Engineering, Engineering Campus, Universiti Sains Malaysia, 14300, Nibong Tebal, Pulau Pinang, Malaysia.

[3]NanoBiotechnology Research & Innovation (NanoBRI), Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, 11800, Pulau Pinang, Malaysia.

[4]Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, Kepala Batas, Penang, 13200, Malaysia

[5]Department of Applied Physics, Faculty of Science & Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia.

[6]Nuclear Technology Research Center, Faculty of Science & Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

*Corresponding Author; e-mail: wnordiana@ukm.edu.my

Received date: 3 March 2025
Revised date: 16 March 2025
Accepted date: 26 May 2025

ABSTRACT

Radiotherapy has been proven to effectively treat cancer along with chemotherapy and surgery. However, some radioresistant and metastasis cancer cells often circumvent the potential efficacy of radiation therapy. The application of radiosensitizer in radiation therapy may potentially inhibit metastasis activation. In this study, the effects of bismuth oxides nanoparticles (BiONPs) on cellular migration in photon beam irradiated cancer cells and the effectiveness of BiONPs to induce radiosensitization were investigated. BiONPs (60nm) with concentration of 0.5 µMol/L were incubated with the HeLa cells prior to irradiation with 6 MV photon beam. The cellular migration test was conducted on the irradiated cells and the total count of the cell migrated was visualized and measured using Image Pro Plus Software. The radiosensitization effects were also validated by measuring the production of reactive oxygen species (ROS). The BiONPs were observed to influence the cellular migration on irradiated HeLa cells. The irradiated cells with BiONPs show a lower total count of migrated cells in contrast to the treatment without BiONPs shows a higher total count of migrated cells with cells count of 23 and 66 respectively at 3 hours incubation time. The ROS results supported the presence of radiosensitization effects induced by the BiONPs with increasing ROS ratio from 1.5 without BiONPs to 1.9 in the presence of BiONPs. The BiONPs are proven to influence the cellular migration in HeLa cells irradiated with 6 MV photon beam. Less cells are found to migrate when BiONPs are applied. ROS production also increased which indicates radiosensitization effects induced by BiONPs. The application of BiONPs in cancer radiation therapy could increase tumour cells' radiosensitivity and lead to prevent cancer metastasis in the future.

Keywords: Bismuth oxide nanoparticles, cellular migration, radiosensitization, photon beam radiotherapy, reactive oxygen species

ABSTRAK

Terapi sinaran telah terbukti berkesan untuk merawat kanser bersama dengan kemoterapi dan pembedahan. Namun begitu, kewujudan sel kanser yang radioresistan dan berkeupayaan bermetastasis sering kali mengurangkan keberkesanan potensi terapi sinaran. Penggunaan bahan pemeka sinaran (radiosensitizer) dalam terapi sinaran berpotensi untuk menghalang pengaktifan metastasis. Dalam kajian ini, kesan nanopartikel oksida bismut (BiONPs) terhadap migrasi sel dalam sel kanser yang disinari dengan pancaran sinar foton, serta keberkesanan BiONPs dalam mendorong pemekaan sinaran, telah dikaji. BiONPs (60 nm) pada kepekatan 0.5 μMol/L telah diinkubasi bersama sel kanser HeLa sebelum diradiasi dengan sinar foton 6 MV. Ujian migrasi sel telah dijalankan ke atas sel yang diradiasi, dan jumlah keseluruhan sel yang bermigrasi telah divisualkan dan diukur menggunakan Perisian Image Pro Plus. Kesan pemekaan sinaran juga disahkan melalui pengukuran penghasilan spesis oksigen reaktif (ROS). BiONPs didapati mempengaruhi migrasi sel pada sel kanser HeLa yang telah diradiasi. Sel yang diradiasi bersama BiONPs menunjukkan jumlah migrasi sel yang lebih rendah berbanding rawatan tanpa BiONPs, dengan kiraan sel masing-masing sebanyak 23 dan 66 selepas 3 jam masa inkubasi. Keputusan ROS turut menyokong kehadiran kesan pemekaan sinaran yang dicetus oleh BiONPs, dengan peningkatan nisbah ROS daripada 1.5 tanpa BiONPs kepada 1.9 dengan kehadiran BiONPs. BiONPs terbukti mempengaruhi migrasi sel kanser HeLa yang diradiasi dengan sinar foton 6 MV. Bilangan sel yang bermigrasi didapati berkurangan apabila BiONPs digunakan. Penghasilan ROS juga meningkat, yang membuktikan kesan pemekaan sinaran yang diinduksi oleh BiONPs. Penggunaan BiONPs dalam terapi sinaran kanser berpotensi meningkatkan kepekaan radiosensitiviti sel tumor dan seterusnya membantu mencegah metastasis kanser pada masa akan datang.

Kata kunci: Nanopartikel oksida bismut, Migrasi sel, Pemekaan sinaran, Radioterapi Foton, Spesis oksigen reaktif (ROS)

INTRODUCTION

Radiotherapy is an essential therapeutic modality for cancer treatments. Most commonly cancer cells are killed during several cycles of radiotherapy. Surprisingly, some cancers may develop radioresistance, migration, and invasion after radiation treatment, which leads to recurrence and metastasis (Su et al. 2012; Barker et al. 2015; Zhang et al. 2023). This unpredictable behaviour of cancer cells, proliferate from the prime tumour to other neighbouring normal tissues and organs of the body where they create new tumours, precedes over 90% of all cancer deaths (Mehlen & Puisieux 2006; Steeg 2006; Fouad & Aanei 2017).

Narrowing the treatment hurdle with radiotherapy alone, there is a critical attention needed to prevent metastasis and invasion regimens (Ran 2015; Weber 2013; Volk-Draper et al. 2014). A strategic investigation in the metastatic phenomenon is cell migration (Suresh 2007; Schuessler et al. 2014). Cell migration propagates invasion, the critical step in tumour spreading. Therefore, tactics that cease cell migration are crucial in improvising treatments that will promise a better outcome for cancer patients (Weber 2013; Gotzer 2016).

Recent studies have shown that radiotherapy in combination with radiosensitizer exhibits the anti-resistance behaviours in the cancer cells. The implication of radiosensitizer has been widely investigated and metallic nanoparticles (MNPs) have been experimentally proven to increase cancer cell damage, due to its high atomic number (Choi et al. 2020; Haque et al. 2023). The presence of MNPs would help to increase the chance of radiation interaction within the tumour as a target, hence will amplifying damage toward targeted cancer cells (Mansouri et al. 2023). The special characteristics of nanoparticles itself possess distinctive size-dependant features, high surface interaction, tuneable and biocompatible (Tremi et al. 2021). Several pre-clinical investigations combining radiotherapy with radiosensitizers such as gold nanoparticles (AuNPs), platinum nanodendrites (PtND), and bismuth oxide nanoparticles (BiONP) had depicted promising outcomes (Jamil et al. 2021, Sisin et al. 2019, Muhammad et al. 2018, Rahman et al. 2014).

Among different types of MNPs, bismuth-based nanoparticles were found to enhance radiation dose more than AuNPs (Algethami et al. 2015; Shahbazi et al. 2020). This is due to the characteristic of higher atomic number (Z=83) and bismuth nanoparticles could elevate the interaction of radiation for both low and high radiation energies combined (Stewart et al. 2016). Reactive oxygen species (ROS) generation was found to be boosted to almost 100% compared to control samples (without BiONPs) when irradiated with photon and electron beams in the presence of BiONPs. (Jamil et al. 2021, Sisin et al. 2019, Sisin et al., 2023, Abidin et al., 2019, Sisin et al., 2020).

Augmenting damage to the cancer cells while preventing further metastasis is the goal of this current study. In this study, BiONPs were investigated as potential radiosensitizers that can also be employed to inhibit cancer cells' migration ability. Cell migration assay was employed to track the cell metastasis ability by comparing the

treatment with and without bismuth oxides nanoparticles (BiONPs). The radiosensitization effects were quantified through the production of ROS generation using 6 MV photon beam.

MATERIALS AND METHODS

BISMUTH OXIDE NANOPARTICLES (BiONPs) PREPARATION

The BiONPs of 60 nm were synthesized using hydrothermal method that has been described in the previous study (Zulkifli et al 2017, Zulkifli et al., 2018). Characterization of the nanoparticles has also been reported in previous studies. These BiONPs were rod-shaped with length average of 500 nm. The powder form BiONPs were diluted in complete cell culture media of Dulbecco's Modified Eagle Medium (DMEM, Life Technologies, U.S.A) supplemented with 10% fetal bovine serum (FBS, Life Technologies, U.S.A) and 1% penicillin streptomycin (Life Technologies, U.S.A).

CELL PREPARATION

HeLa cell lines were obtained from the American Type Culture Collection (ATCC ®). The cells were grown in T75 cell culture flask with complete media of Dulbecco's Modified Eagle Medium (DMEM, Life Technologies, U.S.A) supplemented with 10% fetal bovine serum (FBS, Life Technologies, U.S.A) and 1% penicillin streptomycin (Life Technologies, U.S.A) until reaching 90% confluence at the temperature 37oC with 5% of carbon dioxide (CO2) in humidified atmosphere in the cell's incubator. The cells were washed with phosphate buffer saline (PBS) then harvested with 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) and later re-cultured in 6 wells plates for scratch assay. The cells for ROS measurement were prepared in suspension for irradiation.

SCRATCH ASSAY

In this study, the cells were seeded in 96-wells plates at approximately 1000 cells count/well. Each of the samples was labelled as treated cells with BiONPs and Control for cells without BiONPs. The cell samples were incubated for 24 hours and irradiated with 6 MV photon beams then followed with a scratching procedure using 200µ of micropipette end tip. The fresh complete media were replaced for each sample before irradiation. Post-irradiation, the images of the scratch area were taken for 0 hours, 2 hours, and 3 hours using a Leica inverted microscope. Image Pro Plus software was used to count and record the number of cell migrations within the scratch area.

REACTIVE OXYGEN SPECIES (ROS) MEASUREMENT.

The generation of ROS was measured to quantify the magnitude of radiosensitization effects by BiONPs. The 2-7- dichlorodihydrofluorescein diacetate (DCFH-DA) solution from Sigma–Aldrich (St. Louis, MO, USA) was used as the reagent to detect ROS production. Approximately 5000 HeLa cells were seeded in 96 well plates. Each of the wells was treated with BiONPs and ROS reagents. Before irradiation, the ROS generations were evaluated under fluorescence mode using a microplate reader with an excitation wavelength of 485 nm and an emission wavelength of 525 nm. Then, the cell was irradiated with 1.5 Gy of 6 MV photon beam, and the ROS measured again immediately for post-irradiation. The ROS measurements were then repeated after 1 hour, 2 hours, and 3 hours post-irradiation. ROS ratio was calculated by taking the ratio of the irradiated cell's ROS with or without nanoparticles divided by the non-irradiated cell's ROS value.

IRRADIATION SETUP

The well plate containing cell samples was placed on top of a solid water phantom of 30 cm x 30 cm with a total thickness of 15 cm which represented average human pelvis thickness. The irradiation was set up at the source to surface distance (SSD) of 100 cm with the field size opening by 10 x 10 cm2. The bolus with a thickness of 1.5 cm was positioned on top of the sample to deliver the maximum dose to the cells, in which the specific depth for 6 MV photon irradiation calibration set up. The dose delivered for each sample was 1.5 Gy (150 MU). The samples were irradiated using Elekta Synergy® linear accelerator (LINAC) at the Radiotherapy Unit, Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia (USM). The irradiation set up is presented in Figure 1.

RESULTS AND DISCUSSION

SCRATCH ASSAY

Figure 2 shows the scratch test results recorded at different incubation times from 0 to 3 hours after irradiation with 6 MV photon beams. The images in Figures 2 A, B, and C visualize the cell migration for control samples while Figures 2 D, E, and F are the images for samples treated with BiONPs. At the scratch area, less cell migration was observed at 0 hours. Then after 2 hours of incubation, the cells were started to fill in the empty area aggressively. After 3 hours of cell incubation, the cell migration had fulfilled the gaps, and the number of cells increasingly multiplied. The results indicate

delayed primary cancer cell growth and migration even after delivery of radiotherapy dose (Zhang et al., 2023). Cancer cells after irradiation had a strong ability to promote the entry of monocyte-myeloid-derived suppressed cells (M-MDSCs), which played a significant role in immune suppression and contributed to cancer progression. M-MDSCs could infiltrate into the lung to form a pre-metastatic niche, which increases the risk of cancer cells spreading in the lung by promoting cancer cell migration and inhibiting T cell function (Zhang et al. 2023)

Figure 2: Post-irradiation for scratch test. Images A, B, and C visualized the cell migration for the Control sample. While the D, E, and F images for the sample treated with BiONPs, both samples tested at the same time interval (0 hours, 2 hours, and 3 hours, respectively). However, Figures 2 D, E, and F show images of the scratch area for the samples treated with BiONPs with different results. At 0-hour incubation, no obvious cell migration was seen between the gaps. After 2 hours of incubation, cells grow deliberately filling the empty area. After 3 hours, cells started to proliferate slowly and migrate between the gaps. The combination treatment inhibited the cells' migration progress, as fewer cells filled in the gap compared to irradiation alone. Salah et al. reported that the combined treatment had proven to down-regulated the levels of EMT-associated proteins which regulated cell polarity, cell adhesion, migratory and invasive capacity. Experiments on an aggressive type of pancreatic cancer cell enriched in CSCs demonstrated the ability of titanium peroxide nanoparticles to sensitize difficult-to-treat cells of cancer to radiation therapy (Salah et al. 2022). The result of the cell samples without BiONPs after irradiation shows a higher cell migration rate compared to samples with BiONPs. The total count of the cells that migrate to the scratch area is shown in Table 1.

Table 1 presents the increase of migrated cell numbers with incubation time from 0 to 3 hours. The number of cells accumulated from 43 to 66, respectively for samples without BiONPs. In contrast, samples with BiONPs show decrement over time of incubation. The count number of cell migrations depleted from 38 to 35 and lastly 23 for 0 to 2 and 3 hours incubation respectively.

REACTIVE OXYGEN SPECIES (ROS) MEASUREMENT.

BiONPs increase the probability of radiation interaction especially the secondary radiation within the vicinity of cancer cells. Secondary radiation generates ROS which could increase the incident probabilities that contribute to the damage of cancer cell's DNA. Figure 3 shows the increment of ROS generation due to BiONPs in comparison to the control sample without BiONPs. The ROS results show an increasing ROS ratio from 1.1, 1.3, and 1.5 without BiONPs to 1.3, 1.6, and 1.9 in the presence of BiONPs at 1,2 and 3 hours post-irradiation respectively. The conversion of 2'7'-dichlorofluorescin (DCFH) to 2'7'-dichlorofluorescein (DCF) after irradiation triggers chain reactions by the fluorescence signal increasing continuously before coming to an end. This result was in concurrence with other studies that reported the effect of incubation time as the ROS values increase with time up to 3.5 hours before it starts gradually decrease later on (Sisin et al. 2020). Figure 3: The ROS ratio with and without BiONPs at different radiation doses irradiated with 6 MV photon beam

Intercellular ROS are found to induce strong DCF fluorescence signals when irradiated with titanium peroxide nanoparticles (Salah et al. 2022). Increased intercellular ROS generation when combining both nanoparticles and radiation therapy imparted a complete inhibition of tumour growth (Salah et al. 2022). Deng and colleagues measured around six-fold ROS increment towards treated cancer cell samples with modified BiONPs when compared to untreated ones (Deng et al. 2018). Another study investigated BiONPs with HEK293 cells and found that the production of ROS has induced the formation of double-membrane vacuoles which determines of autophagy process (Liu et al. 2018). This study indicates an increased detrimental effect on the cancer cells when nanoparticles are applied during radiation treatment.

The production of ROS due to the BiONPs validates the occurrence of radiosensitization effects. A study based on simulation using GATE-9.0 and Geant4-DNA on the event of post-irradiation of gold nanoparticles has proven many secondary irradiations of low-energy electrons are produced by the contribution of low energy Auger electrons and photoelectrons rain, originated from Compton interactions with the nanoparticles, thus deposited therapeutic effects towards close surrounding area of targeted cancer region (Bardane et al. 2024). The accumulation of secondary proactive reactions may significantly cause intense radiation deposition in or close to the cancer intracellular. The interaction may locally eradicate the cancer cells while sparing neighbouring normal tissue. Hence less probability of recurrent incidents of metastasis (Remita & Lampre 2024).

Furthermore, studies have proved that bismuth nanoparticles have elevated the radiosensitization effects towards 4T1 breast cancer cells with 2 Gy, 4 Gy, and 6 Gy prescribed doses of X-ray irradiation (Abhari et al. 2020). The effects of radiation dose enhancement by bismuth nanoparticles were observed following irradiation with gamma

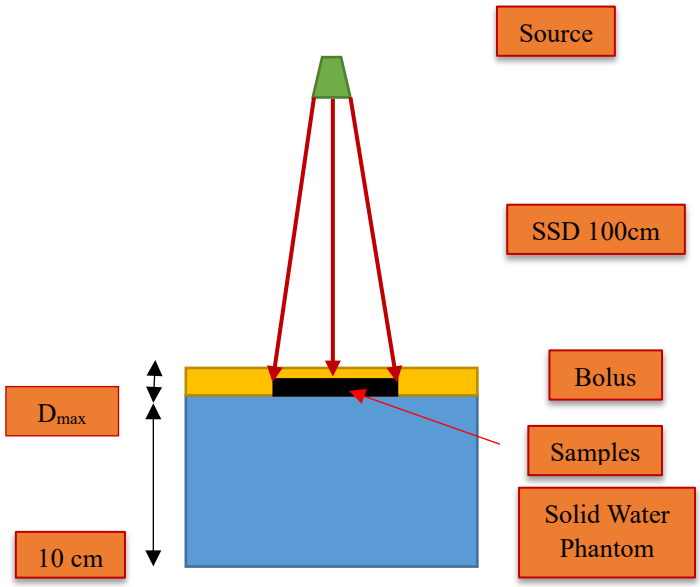

FIGURE 1 The schematic illustration of cells' irradiation setup with 6 MV photon beam

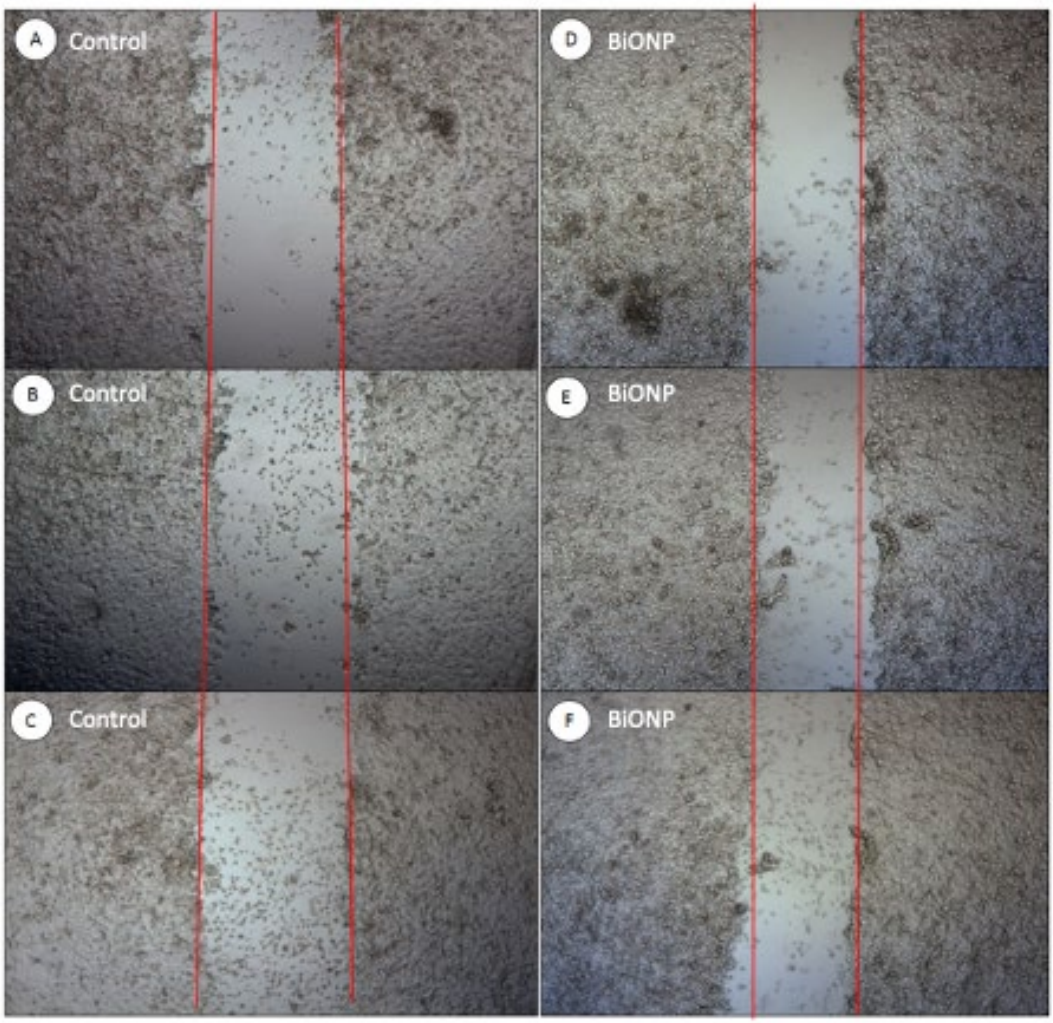

FIGURE 2 Post-irradiation for scratch test. Images A, B, and C visualized the cell migration for the Control sample. While the D, E, and F images for the sample treated with BiONPs, both samples tested at the same time interval (0 hours, 2 hours, and 3 hours, respectively)

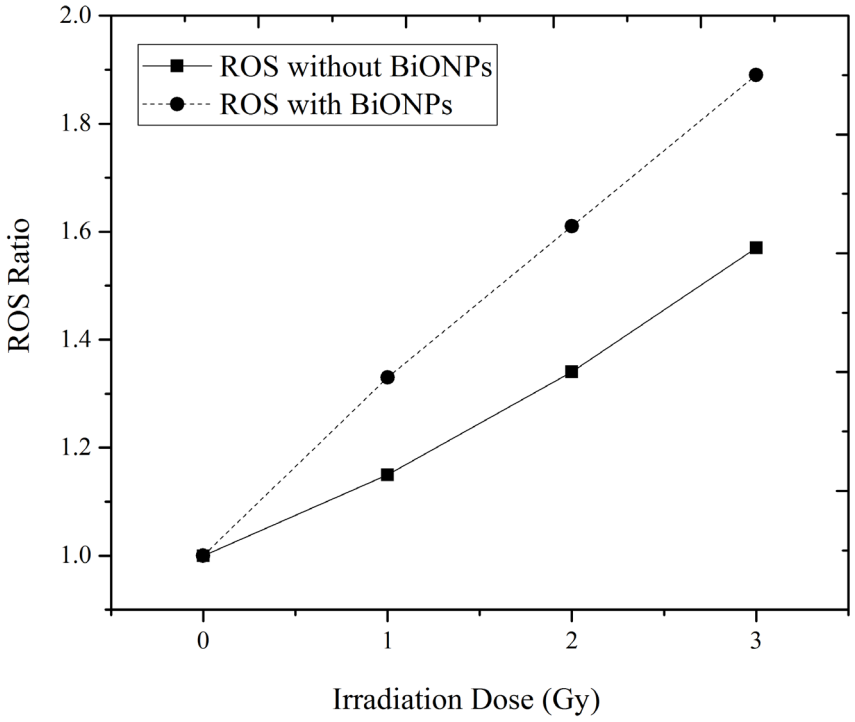

FIGURE 3 The ROS ratio with and without BiONPs at different radiation doses irradiated with 6 MV photon beam.

TABLE 1. The total count of cells migrated in the scratch area with and without BiONPs after irradiation with 6 MV photon beams.

Samples	Time (hours)	Total Count
Control	0	43
	2	55
	3	66
BiONPs	0	38
	2	35
	3	23

radiation from an iridium-192 source of high-dose-rate brachytherapy (Farahani et al. 2020; Sisin et al., 2023). Cell damage presumed by this ROS reaction may support the potential BiONPs application in radiotherapy. This shows that the growth of HeLa cells declined and emptied the gaps while actively producing the ROS in the presence of BiONPs. The usage of BiONPs in cancer radiation therapy could increase tumour cells' radiosensitivity and lead to prevent cancer metastasis in the future.

CONCLUSION

Our findings accentuate the potential of BiONPs in eradicating cancer cells resonates with previously published studies. The integration of BiONPs as radiosensitizers enhanced antitumor activity and diminished migration growth. This study may serve as a stepping stone by exemplifying the next stage of investigation. A strong foundation of this in vitro exploration and further in vivo investigations are pivotal to reaffirming the efficacy of BiONPs in cancer radiotherapy.

REFERENCE

Abhari, F., Charmi, J., Rezaeejam, H., Karimimoghaddam, Z., Nosrati, H., Danafar, H. & Farajollahi, A. 2020. Folic acid modified bismuth sulfide and gold heterodimers for enhancing radiosensitization of mice tumors to X-ray radiation. *ACS Sustainable Chemistry & Engineering* 8(13): 5260-5269.

Abidin, S.Z., Razak, K.A., Zin, H., Yunus, M.A., Wail, N.H.N., Ab Rashid, R. & Rahman, W.N., 2019. Comparison of clonogenic and PrestoBlue assay for radiobiological assessment of radiosensitization effects by bismuth oxide nanorods. *Materials Today: Proceedings* 16: 1646-1653.

Algethami, M., 2018. Bismuth-based nanoparticles as theranostic agents for x-ray Computed Tomography (CT) and radiation therapy (Doctoral dissertation, RMIT University).

Bardane, A. & Maalej, N., 2024. Gold nanoparticle effect on dose and DNA damage enhancement in the vicinity of gold nanoparticles. *Nuclear Analysis* 3(4):1-11.

Barker, H.E., Paget, J.T., Khan, A.A. & Harrington, K.J. 2015. The tumour microenvironment after radiotherapy: mechanisms of resistance and recurrence. *Nature Reviews Cancer* 15(7): 409-425.

Choi, J., Kim, G., Cho, S.B. & Im, H.J., 2020. Radiosensitizing high-Z metal nanoparticles for enhanced radiotherapy of glioblastoma multiforme. *Journal of Nanobiotechnology* 18: 1-23.

Deng, S., Zhou, J.L., Fang, H.S., Nie, Z.G., Chen, S. & Peng, H., 2018. Sesamin protects the femoral head from osteonecrosis by inhibiting ROS-induced osteoblast apoptosis in rat model. *Frontiers in physiology* 9:1-8.

Farahani, S., Alam, N.R., Haghgoo, S., Shirazi, A., Geraily, G., Gorji, E. & Kavousi, N., 2020. The effect of bismuth nanoparticles in kilovoltage and megavoltage radiation therapy using magnetic resonance imaging polymer gel dosimetry. *Radiation Physics and Chemistry* 170:1-8

Fouad, Y.A. & Aanei, C., 2017. Revisiting the hallmarks of cancer. *American journal of cancer research* 7(5):1016-1036.

Gotzer, M.A., Neve, A. & Baumgartner, M., 2016. Dissecting brain tumor growth and metastasis in vitro and ex vivo. *Journal of cancer metastasis and treatment* 2016(2): 149-162.

Haque, M., Shakil, M.S. & Mahmud, K.M., 2023. The promise of nanoparticles-based radiotherapy in cancer treatment. *Cancers,* 15(6):1-25.

Jamil, A., Abidin, S.Z., Razak, K.A., Zin, H., Yunus, M.A. & Rahman, W.N., 2021. Radiosensitization effects by bismuth oxide nanorods of different sizes in megavoltage external beam radiotherapy. *Reports of practical oncology and radiotherapy: journal of Greatpoland Cancer Center in Poznan and Polish Society of Radiation Oncology*, 26(5): 773-784.

KA, M.A., Ab Rashid, R., Lazim, R.M., Dollah, N., Razak, K.A. & Rahman, W.N., 2018. Evaluation of radiosensitization effects by platinum nanodendrites for 6 MV photon beam radiotherapy. *Radiation Physics and Chemistry*150: 40-45.

Liu, Y., Yu, H., Zhang, X., Wang, Y., Song, Z., Zhao, J., Shi, H., Li, R., Wang, Y. & Zhang, L.W., 2018. The protective role of autophagy in nephrotoxicity induced by bismuth nanoparticles through AMPK/mTOR pathway. *Nanotoxicology* 12(6): 586-601.

Mansouri, E., Mesbahi, A., Hejazi, M.S., Montazersaheb, S., Tarhriz, V., Ghasemnejad, T. & Zarei, M., 2023. Nanoscopic biodosimetry using plasmid DNA in radiotherapy with metallic nanoparticles. *Journal of applied clinical medical physics* 24(2):1-13.

Mehlen, P. & Puisieux, A., 2006. Metastasis: a question of life or death. *Nature reviews cance*r 6(6): 449-458.

Rahman, W.N., Corde, S., Yagi, N., Abdul Aziz, S.A., Annabell, N. & Geso, M., 2014. Optimal energy for cell radiosensitivity enhancement by gold nanoparticles using synchrotron-based monoenergetic photon beams. *International journal of nanomedicine*: 2459-2467.

Ran, S., 2015. The role of TLR4 in chemotherapy-driven metastasis. *Cancer research* 75(12): 2405-2410.

Remita, H. & Lampre, I., 2024. Synthesis of Metallic Nanostructures Using Ionizing Radiation and Their Applications. *Materials* 17(2):1-23.

Salah, M., Akasaka, H., Shimizu, Y., Morita, K., Nishimura, Y., Kubota, H., Kawaguchi, H., Sogawa, T., Mukumoto, N., Ogino, C. & Sasaki, R., 2022. Reactive oxygen species-inducing titanium peroxide nanoparticles as promising radiosensitizers for eliminating pancreatic cancer stem cells. *Journal of Experimental & Clinical Cancer Research* 41(1):1-21.

Schuessler, T.K., Chan, X.Y., Chen, H.J., Ji, K., Park, K.M., Roshan-Ghias, A., Sethi, P., Thakur, A., Tian, X., Villasante, A. and Zervantonakis, I.K., 2014. Biomimetic tissue–engineered systems for advancing cancer research: NCI strategic workshop Seyfried, T.N. & Huysentruyt, L.C., 2013.

On the origin of cancer metastasis. *Critical Reviews™ in Oncogenesis* 18(1-2): 5359–5363.

Shahbazi-Gahrouei, D., Choghazardi, Y., Kazemzadeh, A., Naseri, P. & Shahbazi-Gahrouei, S., 2023. A review of bismuth-based nanoparticles and their applications in radiosensitising and dose enhancement for cancer radiation therapy. *IET nanobiotechnology* 17(4):302-311.

Sisin, N.N.T., Abidin, S.Z., Yunus, M.A., Zin, H.M., Razak, K.A. & Rahman, W.N., 2019. Evaluation of bismuth oxide nanoparticles as radiosensitizer for megavoltage radiotherapy. *Int J Adv Sci Eng Inf Technol*, 9(4): 1434-1443.

Sisin, N.N.T. & Rahman, W.N., 2023. Potentials of Bismuth-Based Nanoparticles and Baicalein Natural Compounds as Radiosensitizers in Cancer Radiotherapy: a Review. *BioNanoScience* 13(1):300-321.

Sisin, N.N.T., Azam, N.A., Rashid, R.A., Abdullah, R., Razak, K.A., Abidin, S.Z. and Rahman, W.N., 2020, March. Dose enhancement by bismuth oxide nanoparticles for HDR brachytherapy. *In Journal of Physics: Conference Series* 1497(1) IOP Publishing.

Steeg, P.S., 2016. Targeting metastasis. *Nature reviews cancer* 16(4): 201-218.

Su, W.H., Chuang, P.C., Huang, E.Y. & Yang, K.D., 2012. Radiation-induced increase in cell migration and metastatic potential of cervical cancer cells operates via the K-Ras pathway. *The American journal of pathology* 180(2): 862-871.

Suresh, S., 2007. Biomechanics and biophysics of cancer cells. *Acta biomaterialia* 3(4): 413-438.

Tremi, I., Spyratou, E., Souli, M., Efstathopoulos, E.P., Makropoulou, M., Georgakilas, A.G. & Sihver, L., 2021. Requirements for designing an effective metallic nanoparticle (NP)-boosted radiation therapy (RT). Cancers, 13(13):1-28.

Volk-Draper, L., Hall, K., Griggs, C., Rajput, S., Kohio, P., DeNardo, D. & Ran, S., 2014. Paclitaxel therapy promotes breast cancer metastasis in a TLR4-dependent manner. Cancer research, 74(19): 5421-5434.

Weber, G.F., 2013. Why does cancer therapy lack effective anti-metastasis drugs? *Cancer letters* 328(2): 207-211.

Zhang, H., K. Cao, J. Xiang, M. Zhang, M. Zhu, and Q. Xi. "Hypoxia Induces Immunosuppression, Metastasis and Drug Resistance in Pancreatic Cancers." *Cancer Letters* 571 (2023): 216345.

Zulkifli, Z. A., K. A. Razak, and W. N. W. A. Rahman. 2017. "Effect of Hydrothermal Reaction Time on Size of Bismuth Oxide Nanoparticles Synthesized via Hydrothermal Method." *AIP Conference Proceedings* 1901 (1). AIP Publishing.

Zulkifli, Z. A., K. A. Razak, W. N. W. A. Rahman, and S. Z. Abidin. 2018. "Synthesis and Characterisation of Bismuth Oxide Nanoparticles Using Hydrothermal Method: The Effect of Reactant Concentrations and Application in Radiotherapy." *Journal of Physics: Conference Series 1082:* 012103. IOP Publishing.