Dose enhancement by bismuth oxide nanoparticles for HDR brachytherapy

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Abstract. Escalation of biological damage through the induction of dose enhancement effect in radiotherapy cancer treatment by high-Z nanoparticles (NPs) has recently been the subject of growing interest. Hence, this study was conducted primarily to investigate the enhancement of brachytherapy (source Ir¹⁹²) efficacy by bismuth oxide nanoparticles (BiONPs) on cervical cancer cells. Radiosensitization effect was tested against different size and concentration of BiONPs. After irradiation with radiation doses ranging from 0 to 4 Gy, the survival of the cell was quantified by clonogenic assay and presented in survival curves fitted using the LQ model. Dose enhancement (DE) factor was extrapolated from the curve at 50% of cell survival and calculated. The results marked out the dependency of DE on nanoparticles size and concentrations. The optimum size of BiONPs was found to be 80 nm with a concentration of 0.00025 mM, in which the DEF is 1.88. In conclusion, this study suggests that the induction of the DE effect is dependent on the size and concentration of the nanoparticles.

1. Introduction

Radiotherapy had become one of the essential treatments for cancer patients. Regardless of the technological advancement to produce the conformal beam, the ionizing radiations that are used to kill the cancer cells may also damage the surrounding healthy tissues. Recent studies have investigated the application of metallic nanoparticles as radiosensitizers or dose enhancement agents that might improve the radiation effects targeted towards the cancer cells only [1].

The efficacy of high atomic number (Z) nanoparticles as an active radiosensitizer for localized tumor has been extensively explored based on high X-ray absorption cross-section of the metallic nanoparticles compared to the biological tissue. The presence of nanoparticles in the tumor would increase the radiation absorption and concentrated more radiation dose at the target site, thus contributed to the DNA damage of the cancer cells [1]. Bismuth element with a high atomic number (Z=83) is considered as a potential candidate to induce dose enhancement (DE) effect, which could increase the brachytherapy efficacy. Bismuth oxide nanoparticles (BiONPs) exhibits favorable characteristic as dose enhancement agents or radiosensitizer as it demonstrated a higher effect in comparison to other types of nanoparticles [2]. This study aimed to evaluate the dose enhancement potentials of BiONPs for brachytherapy on cervical cancer cells.
2. Materials and Methods

2.1. Cells Preparation
HeLa cells (human cervical cancer) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) media (Gibco), with 5% fetal bovine serum (Gibco) and 1% penicillin-streptomycin (Gibco). The cells were grown in 75 ml and 25 ml flask (SPL Life Science, South Korea) until confluence at 37°C and 5% CO₂ humidified atmosphere (CO₂ incubator, Galaxy, Eppendorf).

2.2. Bismuth Oxide Nanoparticles Preparation
Bismuth oxide nanoparticles were synthesized in the form of rod-shaped nano-powder and were suspended in DMEM media. The study was carried out by treating HeLa cells with 70 nm and 80 nm of BiONPs at three different concentrations of 0.0005, 0.00025, and 0.00005 mM. The nanoparticles have been tested for their toxicity before being applied for irradiation.

2.3. Brachytherapy Irradiation
Cells irradiation was performed using Microselectron HDR Brachytherapy machine (Nucletron Corp, Columbia, Maryland) with an iridium-192 source at Hospital Universiti Sains Malaysia. The control cells without BiONPs, and the cells treated with BiONPs were plated in 6-wells-plates (SPL Life Science, South Korea). The plate was arranged on top of the surface mould and covered with bolus. The radiation doses applied were ranged from 0 to 4 Gy. After irradiation, the cells were incubated at 37°C with 5% of CO₂ for a week.

2.4. Clonogenic Assay
The control and irradiated cells were seeded in 6-well plates (1000 cells per well) and incubated at 37°C with 5% of CO₂ for a week until its form colonies. Then, the cells were washed with phosphate buffer saline, followed by fixation with ice-cold methanol for 15 minutes at room temperature. The cells were stained with 3-4 drops of crystal violet for an hour. The stained cells were washed gently with tap water, and the cells colonies were counted.

2.5. Cell Survival Measurement
Survival fractions (SF) were calculated from the ratio of colony formation of irradiated cells to non-irradiated control cells. The SF are presented as the mean ± standard deviation from two independent experiments. The LQ model was employed to fit the experimental data. Enhancement of radiation dose was quantitatively explicated by dose enhancement factor (DEF). The DEF was computed by taking the ratio of dose that produced 50% of the cell survival fraction for control cells against the dose that produced 50% of cell survival fraction for cells with the presence of BiONPs. From the LQ fittings, α and β values corresponded to the linear and quadratic components, which would indicate single hit and double hits of DNA double-strand breakages, respectively [3].

3. Results and Discussion
The survival curves in Figure 1 shows the reduction in surviving fraction of cells treated with BiONPs for every size when compared to the control cells. DE effect was expressed in term of DEF₅₀ as reported in Table 1.

Overall, the DE effects of BiONPs were dependent on the sizes and concentrations. Notably, DEF₅₀ calculated shows that concentration of 0.00025 mM gave the highest enhancement effect for all sizes with values of 1.82 Gy and 1.88 Gy for 70 nm and 80 nm of BiONPs, respectively. A DEF of 1.0 refers to 0% enhancement, whereas a DEF of 2.0 refers to 100% enhancement [4]. A Monte Carlo simulation study showed that 70 mg/g of BiONPs could deliver a DEF up to 18.55 to soft brain tissue [5]. Our previous study had also found that the 60 nm of BiONPs were dose-dependent for photon and electron beam therapies [3]. However, when the concentration of BiONPs in this study was increased to 0.0005 mM, there is a reduction in DEF values for both sizes of BiONPs. This condition might
occur due to the limitations of averaging dose-modifying effect when too many nanoparticles [5]. The DEF could eventually approach a saturation because of the increase in several photons interacting with the tumor volume becomes more insignificant as the BiONPs concentration increases [5].

Next, the DEF calculation also shows dependency on nanoparticles sizes. Several previous studies also support this finding. A Monte Carlo study in brachytherapy using gold NPs for Ir-192 with a diameter of 50, 100, and 200 nm found the DEF increase from 1.10, 1.16, and 1.20, respectively [6]. It was explained that the enhancement was due to Auger production by nanoparticles. The percentage of electrons emitted from nanoparticle upon X-ray excitation strongly depends on particle size, with a majority of low-energy and short-range Auger electrons being absorbed more readily within nanoparticle [4].

**Table 1.** DEF values of 70 nm and 80 nm of BiONPs. Each size consists of 0.00005 mM, 0.00025 mM and 0.0005 mM of concentrations

| Concentration (mM) | DEF | α (Gy⁻¹) | β (Gy⁻²) |
|--------------------|-----|----------|----------|
| 70 nm              |     |          |          |
| Control            | 1.00| 0.27 ± 0.09| 0.02 ± 0.03|
| 0.00005            | 1.23| 0.05 ± 0.16| 0.17 ± 0.07|
| 0.00025            | 1.82| 0.36 ± 0.17| 0.05 ± 0.07|
| 0.0005             | 1.59| 0.19 ± 0.19| 0.11 ± 0.06|
| 80 nm              |     |          |          |
| Control            | 1.00| 0.15 ± 0.04| 0.01 ± 0.01|
| 0.00005            | 1.42| 0.06 ± 0.25| 0.09 ± 0.06|
| 0.00025            | 1.88| 0.24 ± 0.20| 0.06 ± 0.08|
| 0.0005             | 1.42| 0.03 ± 0.06| 0.13 ± 0.02|

**Figure 1.** Survival curves of HeLa cells treated with (a) 70 nm and (b) 80 nm of BiONPs and irradiated with 1 to 4 Gy of brachytherapy.
Quantitative analysis of the cell surviving through LQ formula demonstrated by $\alpha$ and $\beta$ values, as shown in Table 1. The $\alpha$ parameter depicted the most usual cell killing mechanism occurs through a single hit by the radiation, breaking a double strand of the DNA, while $\beta$ component indicates that cells killing mechanism is mostly double hit by radiation at a time or different times [1,3]. In the present study, most of the $\alpha$ values are higher than $\beta$ values, thus suggested that most of the mechanism of actions were caused by a single hit on the double-strand DNA.

4. Conclusions
This study focused on the effect of bismuth oxide nanoparticles (BiONPs) with different size and concentration on brachytherapy efficacy. The particular size of 80 nm BiONPs depicted the highest $\text{DEF}_{50\%}$ than 70 nm. However, further increasing BiONPs concentration resulted in decreasing in $\text{DEF}_{50\%}$ value. The mechanism of the cell death was dominated by single hit double-strand breakages of the cellular DNA, which is represented by the $\alpha$ values. In brief, the enhancement of brachytherapy efficacy on HeLa cells in the presence of BiONPs is validated in this study.

5. References
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