Radiosensitization characteristic of superparamagnetic iron oxide nanoparticles in electron beam radiotherapy and brachytherapy

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Abstract. Superparamagnetic Iron Oxide Nanoparticles (SPIONs) has been the focus in medical imaging as MRI contrast agents. SPIONs demonstrate intriguing properties that not only advantageous in diagnostic imaging but also for therapeutic application. In this study, the radiosensitization characteristic of SPIONs in electron beam radiotherapy and brachytherapy were investigated. This study was conducted in-vitro using HeLa cells with SPIONs (15 nm) concentration of 1, 2 and 3 mMol/L. Irradiations were done at doses ranging from 0 to 10 Gy using electron beams of energy 6 and 12 MeV as well as 0.38 MeV ¹⁹²Ir brachytherapy source. Cell survivals were determined from clonogenic assay. Radiosensitization characterization was performed by analyzing the sensitization enhancement ratio (SER) explicated from the survival curves. The results show SPIONs induce radiosensitization effects in electron beams especially at lower energy with SER value obtained up to 1.5. The radiosensitization is more prominent for brachytherapy with SER value around 2. Concentrations of SPIONs also play important roles in which higher SPIONs concentration are more likely to increase radiosensitization effects. In conclusion, radiosensitization are dependent on radiation energy and concentration of the SPIONs. Further characterization of the radiosensitization induced by SPIONs may enable clinical translation of SPIONs as radiosensitizer in radiotherapy.

1. Introduction
Radiotherapy is one of the important medical techniques to eradicate malignant cells by using ionizing radiation to induce the cancer cells damage and death. High energy radiations such photon, electron and gamma rays that are targeted to deposit dose in the tumour volume can also produces adverse
effects to the surrounding healthy tissue. Therefore, increasing radiation effects on the malignant cells while sparing the normal tissue are the primary goal in radiotherapy.

In the recent years, there were growing evidence on nanoparticles application as radiation dose enhancer or radiosensitizer in radiotherapy [1,2,3,4]. Pioneering work by Hainfeld et al. (2004) on gold nanoparticles has led to more experimental validation on the radiosensitizing properties of different types of nanoparticles for radiotherapy application [1]. Superparamagnetic Iron Oxide Nanoparticles (SPIONs) attracted great attention as potential radiosensitizer in radiotherapy [5]. SPIONs shared similar intriguing features with gold nanoparticles including non-toxic, easy to synthesis, size variation and targeting structural modification [6]. In contrast to gold nanoparticles, SPIONs are relatively more cost effective and retain the unique superparamagnetic properties [7]. SPIONs magnetic characteristic allow them to be directed and localized at specific organ using external magnetic force which make them an excellent magnetic resonance imaging (MRI) contrast agents [8]. Currently, some types of SPIONs have been clinically approved for human application as MRI contrast agents [8]. SPIONs prospect in radiotherapy has been pre-clinically investigated and results indicated the potential of SPIONs as multifunctional agent for cancer diagnostic and therapy [9,10,11,12]. Recent studies also found that SPIONs were effective with proton beam therapy and megavoltage photon beam [13,14,15,16]. In this study, we aimed to investigate the radiosensitization characteristic of SPIONs for electron beam therapy and brachytherapy. Both of this beams type are the parts of standard modality in radiotherapy that is used to treat many types of malignant diseases. This study also examined the effects of different SPIONs concentration and beam energy on the radiosensitization.

2. Materials and Methods

2.1. Nanoparticles and cells preparation
The SPIONs were synthesized using previously reported procedure at the Key Centre for Polymers and Colloids, University of Sydney, Australia [17,18]. The nanoparticles were filtered through a sterile 0.22 μm hydrophilic polysulphonic membrane syringe bacterial filter (Sartorius, Goettingen, Germany) to ensure sterility. SPIONs were diluted to final concentration of 1, 2 and 3 mMol/L. HeLa cell line (ATCC, U.S.A.) were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% fetal bovine serum (Gibco, Life Technologies, City, U.S.A.) and 1% penicillin & streptomycin (Gibco, Life Technologies, City, U.S.A.) at 37°C in a humidified environment of 5% CO₂. The cells were grown to confluence in a 75 cm² flask (SPL Life Sciences, Korea) and split in a ratio of 1:3.

2.2. Cells irradiation
The cell samples were prepared in suspension in 0.5 ml Eppendorf tube (Greiner Bio-One, Austria) with and without SPIONs. The electron beam irradiations (6 and 12 MeV) were performed using Primus Linear Accelerator (Siemens Healthcare, U.S.A) with 10 x 10 cm² electron applicator. The cell samples were set up on a plastic water phantom at the depth of maximum dose (dmax) with source to surface distance (SSD) of 100 cm. Irradiations were done in single fraction with constant dose rate of 100 MU/min with radiation doses ranging from 0 to 10 Gy. The brachytherapy irradiation was performed using Ir-192 source emitting gamma ray of energy 0.38 MeV from Microselectron HDR Brachytherapy V14.23 (Nucletron Corp, Columbia, Maryland). The cell samples were arranged on top of surface mould where the source catheter were placed and covered with bolus. The irradiations were done with dose of 1 to 4 Gy. Gafchromic EBT3 films were used for dose uniformity and geometric validation.

2.3. Clonogenic assay
The irradiated cells were re-cultured in 6 well plates (SPL Life Science, South Korea) and were incubated for a week for colony formation. After incubation, the cells were washed gently with 1 ml Phosphate Buffered Saline (PBS) and were fixed with 500 μl ice cooled methanol. The fixed cells
were then stained with crystal violet and the visible colonies were counted. Survival fractions were calculated and represented by the ratio of colony formation of irradiated cells to non-irradiated control cells. The survival fractions are presented as the mean ± standard deviation from 3 independent experiments.

2.4. Cell survival and data analysis
The survival fraction for samples with and without SPIONs were then plotted and fitted according to the linear quadratic (LQ) model using OriginPro 8.5 software. The parameters of LQ model are shown in the equation (1).

\[ S = \exp^{-(\alpha D + \beta D^2)} \]  

(1)

The sensitization enhancement ratio (SER) were extrapolated from the cell survival curves and calculated by taking the ratio of dose that produces 50% of cell survival fraction for control cells to treated cells with SPIONs as depicted in equation (2).

\[ SER_{50} = \frac{D_{50,\text{control}}}{D_{50,\text{SPIONs}}} \]  

(2)

3. Result and Discussion
The potential cellular damage causes by different concentration of SPIONs with megavoltage electron beam irradiation are portrayed in the cell survival curves shown in Figure 1. Quantitative analysis from the cell survival curves indicates therapeutic effects of SPIONs under irradiation of 6 and 12 MeV electron beams. SER calculated for 1 mMol/L, 2 mMol/L and 3 mMol/L concentration of SPIONS are 1.08, 1.2 and 1.5 respectively for 6 MeV. Higher energy electron beam of 12 MeV are found not to induce radiosensitization effects at lower SPIONs concentration. Enhancements of therapeutic effects are only observed at 3 mMol/L for 12 MeV electron beam. The SER obtained for 1 mMol/L, 2 mMol/L and 3 mMol/L of SPIONS are 0.97, 1 and 1.29 respectively. The cell survival curves for HDR brachytherapy irradiation are displayed in Figure 2. The survival curves present reduction in cell survival for cell with SPIONs compared to the control cell indicating the occurrence of radiosensitization effect for brachytherapy. The SER extrapolated for 1 mMol/L, 2 mMol/L and 3 mMol/L are 1.33, 2 and 1.56 respectively.

Radiobiological analysis of the cell survival curves using LQ model found an increase in \( \alpha \) and \( \beta \) value for every concentration compared to the control. The \( \alpha \) value describe the linear parameter of the curve while \( \beta \) value describe the quadratic parameter of the curve. The \( \alpha \) values obtained for 6 MeV electron beam are 0.17 ± 0.03, 0.2 ± 0.04, 0.24 ± 0.07 and 0.37 ± 0.07 for control, 1 mMol/L, 2 mMol/L and 3 mMol/L, respectively. Meanwhile the \( \beta \) values obtained are 0.03 ± 0.01, 0.03 ± 0.01, 0.04 ± 0.03 and 0.04 ± 0.02 for control, 1 mMol/L, 2 mMol/L and 3 mMol/L, respectively. The parameters for 12 MeV are lower with \( \alpha \) value of 0.02 ± 0.03, 0.03 ± 0.04, 0.07 ± 0.02, 0.17±0.01 and \( \beta \) values of 0.06 ± 0.01, 0.07 ± 0.01, 0.10 ± 0.03 for control, 1 mMol/L, 2 mMol/L and 3 mMol/L, respectively. Higher \( \alpha \) and \( \beta \) values for brachytherapy in comparison to electron beams are observed. In brachytherapy irradiation, the \( \alpha \) value are 0.05 ± 0.04, 0.27 ± 0.04, 0.40 ± 0.14, 0.32 ± 0.03 and the \( \beta \) values are 0.11 ± 0.01, 0.2 ± 0.01, 0.06 ± 0.06 and 0.2 ± 0.02 for control, 1 mMol/L, 2 mMol/L and 3 mMol/L, respectively. In general, the \( \alpha \) parameters for the cell survival curves with SPIONs exhibit higher values compare to control which normally relates to increase in the DNA double strand breakage. The \( \beta \) parameters usually represent single strand breakage are random in value which also lead to variation in \( \alpha/\beta \) parameter [19,20]. Nevertheless the \( \alpha/\beta \) parameter which could be used to indicate the increase radiosensitivity is higher for cells with SPIONs than the control cells. From the results obtained, we can deduce that the radiosensitization effects are highly dependent on beam energy and concentration of the nanoparticles which in agreement with previous study using gold nanoparticles [20].
Figure 1. The cell survival curves as a function of dose for cells irradiated with a) 6 MeV and b) 12 MeV electron beams. The curves were fitted according to LQ model.

Figure 2. The cell survival curves as a function of dose for cells irradiated with HDR brachytherapy with Ir-192 source. The curves were fitted according to LQ model.

Table 1. The SER and radiobiological parameters of LQ model.
4. Conclusion
In conclusion, SPIONs are found to yield radiosensitization effects especially for irradiation with HDR brachytherapy that demonstrate the highest SER value. Irradiation of SPIONs in combination with electron beam also indicates radiosensitization especially at lower energies. The increase of the therapeutic effects of HDR-Brachytherapy and electron beam indicate the potential of SPIONs as radiosensitizer for different radiotherapy modality.

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