

# Use of Poly (Ethylene Glycol) Coated Superparamagnetic Iron Oxide Nanoparticles as Radio Sensitizer in Enhancing Colorectal Cancer Radiation Efficacy

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## Abstract

**Background:** The aim of the radiotherapy is to deliver a lethal dose to tumor while reducing the impact on the normal tissue. This reduction in impact can be achieved to have a greater therapeutic ratio by using nanoparticles as radiosensitizer. **Materials and Methods:** In this article, the potential role of superparamagnetic iron oxide nanoparticles (SPIONs) as radiosensitization enhancer on HT 29 cell lines for different concentrations (0.007 to 0.25 mg/ml) and different radiation doses (0.5 to 2 Gy) of 6MV photon beam is presented. **Results:** The highest sensitization enhancement ratio (SER) value was observed with 2 Gy for 0.25 mg/ml concentration. Radio sensitization increases with increase in the concentration of nanoparticles. Combination of 6MV energy radiation and polyethylene glycol (PEG) coated SPIONs results in increasing cell killing of HT 29 as compared to cell killing with radiation therapy alone. **Conclusion:** The results reveal that PEG coated nanoparticle might be a potential candidate to work as radiotherapy sensitizer in colorectal cancer.

**Keywords:** HT-29 cell lines, radiotherapy sensitizer, superparamagnetic iron oxide nanoparticles

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## INTRODUCTION

Radiotherapy plays an important role in cancer treatment of about 50% cases.<sup>[1]</sup> Most important concern and disadvantage of radiotherapy is that ionizing radiation influences both healthy tissue and solid tumors. To achieve tumor control by increasing radiation doses will damage normal tissues surrounding the target tumor which is a limitation in escalation of dose beyond which radiotherapy cannot be sustainably employed to treat cancers. Thus, there is a need of improvement in radiation delivery techniques so that injury to the surrounding tissues is reduced to achieve better tumor control probability (TCP) successfully.<sup>[2]</sup> Globally, colorectal cancer (CRC) is the third most common type of cancer, making up about 10.2% of all cases.<sup>[3]</sup> In 2018, there were 1.84 million new cases and 880,792 deaths from the disease.<sup>[3]</sup> CRC is more common in developed countries, where more than 65% of cases are found.<sup>[4]</sup> The radiotherapy in rectal cancer has intelligible application from anatomical

perspective as rectum is a relatively fixed structure in the pelvis and it is situated below the organs that have limited tolerance to radiotherapy.<sup>[5]</sup> Conventionally, CRC is treated with surgery in the early stages, while a combination of preoperative chemoradiation therapy (CRT) and surgery is used in the more common locally advanced stages.<sup>[6]</sup> Preoperative CRT results in only about 15% of patients achieving a complete pathological response, i.e., no viable tumor remains within the surgical specimen at the time of surgery.<sup>[7,8]</sup> Furthermore, in lower third rectal cancers with wait and watch policy post-radio-chemotherapy for organ preservation has shown encouraging results.<sup>[9]</sup> In order to achieve greater therapeutic

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ratio, radiation doses can be used to improve tumor down staging and local control of tumors.<sup>[8]</sup> However, the dose escalation also increases the risk of toxicity and exceeds the tolerance of adjacent healthy tissues.<sup>[8]</sup> A better alternative way is to combine standard-dose radiotherapy with radio sensitizers to enhance the radiation therapy efficacy locally within tumor area while saving adjacent healthy tissues.<sup>[6,10]</sup>

Many substances and materials have been reported as radio sensitizers. To propose nanoparticles as novel radio sensitizers, many progresses have been made toward it. The tumor vascularization system is heterogeneous and weak due to having high porosity, being spacious, and high leakage power.<sup>[11]</sup> These factors facilitate nanoparticles passage from the blood to the tumor cells and accumulate there; this is known as enhanced permeability and retention effect.<sup>[11-13]</sup> High atomic number (Z) nanoparticles, such as gold and silver, have been evaluated preclinically in *in vitro* and *in vivo* studies.<sup>[5]</sup> When lower energy photon interacts with high atomic number nanoparticles, photoelectric (PE) interaction becomes dominant. In PE interaction, there will be greater ionization, greater generation of secondary electrons and free radicals thus ultimately leading to greater DNA damages.<sup>[5,14]</sup> Most of studies to investigate the radio sensitization effects of high atomic number (Z) nanoparticles have used kilovolt (kV) energies to get advantage of PE effects which is directly proportional to  $Z^4$ - $Z^{4.6}$ .<sup>[15]</sup> In radiotherapy, megavoltage (MV) photons are more clinically relevant energy radiations to provide skin sparing effect and efficient dose to deeply seated tumors. MV photons interact with matter through Compton scattering, which is less dependent on Z. Other metal-based nanoparticles (where metals such as hafnium oxide<sup>[16]</sup> and iron oxide<sup>[17]</sup>) have also been studied and reported to enhance the therapeutic efficiency of radiotherapy. Amongst nanoparticles, superparamagnetic iron-oxide nanoparticles (SPIONs) are in the intense focus of research because of their several biomedical applications, such as a contrast agent for magnetic resonance imaging, amenability for functionalization with different capping agents, excellent platform for targeted co-delivery of chemotherapeutic drugs, and magnetic hyperthermia therapy of cancer.<sup>[17]</sup> In recent years, a few studies have reported that the radio-sensitizing ability of iron oxide nanoparticles, which was conjectured principally, due to their high surface-to-volume ratio, may act as a catalyst for the generation of reactive oxygen species (ROS).<sup>[17,18]</sup>

Several studies reported that SPIONs were enhancing the effect of radiation on cancer cells. Klein *et al.*,<sup>[19]</sup> demonstrated that citrate-coated SPIONs may function as excellent radiosensitizer upon impact of X-rays in enhancing the generation of ROS about 240% as compared with X-ray treated cells without internalized SPIONs. Coating the SPIONs by a biocompatible material has several advantages such as the prevention of agglomeration among themselves, minimization of unnecessary uptake by reticuloendothelial system and increase in biodistribution of nanoparticles.<sup>[17,20]</sup> Coating plays a peculiar role in cellular internalization and toxicity. These roles have been investigated by Huang *et al.*,<sup>[21]</sup> investigated that iron oxide nanoparticles

with two different surface modifications, namely dextran coating and cross-linked dextran coating show that their different internalization affects their capability to enhance radiation damage of cancer cells. One of the most successful approaches in producing surfaces that is capable to resist protein adhesion and biological attack will be to use polyethylene glycol (PEG) as a surface protector.<sup>[22]</sup> Furthermore, it was demonstrated that both *in vitro* and *in vivo* studies the PEG coating suppresses platelet adhesion, leading to reduced risk of thrombus formation, tissue damage, and other cytotoxic effects.<sup>[22]</sup>

In this study, our aim is to synthesize PEG-coated SPIONs and analyze the cell survival study of colorectal cancer cell lines (HT-29) using PEG-coated SPIONs as a radiosensitizer. The radiation survival of cells has traditionally been measured by clonogenic assay which is established standard but also difficult and time-consuming. Here, we have used the MTT assay to measure radiation cell survival by estimating sensitizer enhancement ratio (SER).<sup>[23]</sup>

## METHODS AND MATERIALS

### Materials

Ferric chloride anhydrous ( $\text{FeCl}_3$ , 96%), ferrous chloride hydrated ( $\text{FeCl}_2$ , 98%), potassium hydroxide pellets (KOH), and PEG 400 were used as purchased without further purification. HT-29 human colorectal cancer cell lines, Dulbecco's Modified Eagle Medium (DMEM), phosphate buffer saline (pH 7.4), 96 well micro titer plate, and cells were maintained in a tissue culture  $\text{CO}_2$  incubator at  $37^\circ\text{C}$  with 5.0%  $\text{CO}_2$ .

### Synthesis of polyethylene glycol coated and uncoated $\text{Fe}_3\text{O}_4$ nanoparticles

PEG coated  $\text{Fe}_3\text{O}_4$  were synthesized by chemical co-precipitation method.<sup>[23]</sup> In a beaker containing 100 ml of distilled water, 2 M of ferric chloride, 1 M of ferrous chloride, and 10 ml of PEG 400 were added and stirred for 30 min. To this 1 M of KOH solution was added drop wise to obtain homogenous solution. Temperature was maintained at  $90^\circ\text{C}$  with stirring rate 1100 rpm. The resultant black precipitate was allowed to settle down. Precipitate was magnetically separated using permanent magnet and washed 3–4 times and kept in oven at  $90^\circ\text{C}$  for 12 h. For synthesis of bare  $\text{Fe}_3\text{O}_4$ , the above procedure was repeated without addition of PEG400 in the bath under nitrogen atmosphere.

### Structural and morphological characterization

The X-ray diffraction patterns of PEG-coated  $\text{Fe}_3\text{O}_4$  nanoparticles were performed on an X-ray Bruker AXS D8 advance diffractometer equipped with source Cu  $\alpha$  radiation ( $\lambda = 1.5406 \text{ \AA}$ ) at the step size  $0.100203^\circ$ . The average crystallite size has been estimated from X-ray diffraction pattern, using the Scherer's equation,  $\beta = K\lambda/d \cos \theta$ .<sup>[24]</sup> Where  $\beta$  is the peak width at half of maximum intensity, K is the shape factor,  $\lambda$  is the wavelength of X-ray ( $\lambda = 1.5406 \text{ \AA}$ ), d is the average crystallite size, and  $\theta$  is the Bragg's angle of diffraction in degree. It should be noted that the shape factor K is dimensionless and is accounting the

shape of the specimen and often has the value of 0.89.<sup>[25]</sup> Size and morphology of PEG coated  $\text{Fe}_3\text{O}_4$  nanoparticles were studied with transmission electron micrograph using JEOL JEM-2100F (Country of origin: Japan) Field Emission Gun Transmission Electron Microscope (HR-TEM).

### Cell culture

*In vitro* growth inhibition effect of test compound was assessed by colorimetric or spectrophotometric determination by conversion of MTT into “Formazan blue” by living cells.  $1 \times 10^5$  cells/ml HT-29 cell suspension was seeded into each well of 96 well micro titer plate and final volume was made up to 150  $\mu\text{l}$  by adding DMEM media and incubated overnight. Dilutions of the test compound, i.e., PEG-coated SPION's were prepared in DMEM media. 100  $\mu\text{l}$  of the test compound with different concentrations of 0.007, 0.015, 0.031, 0.062, 0.125, and 0.25 mg/ml was added to the wells and normal control (cells with medium and no test sample), incubated for 24 h., in presence of 5%  $\text{CO}_2$ , at 37°C into  $\text{CO}_2$  incubator. After incubation, cell culture well plate was exposed to 6 MV X-ray beam under linear accelerator machine and kept for 72 h of incubation. After 3 days, 20  $\mu\text{l}$  of 5 mg/ml MTT reagent was added to the wells. The plate was kept for 4 h of incubation in the dark place at the room temperature. (The plate was covered with aluminum foil, since MTT reagent is photosensitive). The supernatant was carefully removed without disturbing the precipitated Formazan crystals and 100  $\mu\text{l}$  of DMSO was added to dissolve the crystals formed. The optical density (OD) was measured at the wavelength of 492 nm. The study was performed in triplicates, and the result represents the mean of three readings. The surviving fraction of cells was calculated using the formula,  $S.F = \text{Mean OD}_{\text{NP}} / \text{Mean OD}_{\text{CONT}}$

Same experiment has been performed on HT-29 cell lines with 0.007, 0.015, 0.031, 0.062, 0.125, and 0.25 mg/ml concentrations of SPIONs nanoparticles and control cells (cells with medium and no test sample), incubated for 96 hs, in the presence of 5%  $\text{CO}_2$ , at 37°C into  $\text{CO}_2$  incubator. OD was measured without any radiation exposure. Incubation period was kept same as above.



Figure 1: Experimental setup for irradiation of HT29 cell lines

### Irradiation setup

Irradiation of HT29 cell lines was done using MV X-ray beam (6 MV) produced by Varian Clinac iX. Cells were cultured in 96 well plate and incubated for 24 hs with PEG-coated  $\text{Fe}_3\text{O}_4$  nanoparticles of different concentrations. The cell culture plate sandwiched between two solid water phantoms (thickness 5 cm of each) to attain electronic equilibrium and the remaining space filled with tissue equivalent wax bolus, as shown in Figure 1a. Water equivalent material could bring scattered photons of lower energy which interacts by PE effect. In this study, we kept 96 well plate at 5 cm depth instead of  $D_{\text{max}}$  because the energy held by the scattered photon is related to the energy of the incident photon. Therefore, backscattered photons which carry less energy are interesting for X-rays nanoparticles interactions purposes, thus the cells should be located in a low dose gradient: After the depth of the maximum dose and inside the beam, off the penumbra region.<sup>[14]</sup> The cell plates were kept at clinical distance from source, i.e., at 100 cm and 20 cm  $\times$  20 cm field size kept opened, as shown in Figure 1. Irradiation was done for different doses such as 0.5, 1, 1.5, and 2 Gy using anterior-posterior and posterior-anterior parallel opposed technique.

### Statistical analysis

All experiments were carried out in triplicate. The cell survival values presented in the figures show the mean  $\pm$  standard. The cell survival value among the different groups was compared using the two-tailed unpaired *t*-test with the consideration of significant  $P \leq 0.05$ .

## RESULTS

### Structural and morphological characterization

Figure 2 shows X-ray diffraction pattern of (a) without and (b) with PEG coated  $\text{Fe}_3\text{O}_4$  nanoparticles. The diffraction peaks appeared at  $2\theta = 30.92^\circ, 36.33^\circ, 43.84^\circ, 57.77^\circ,$  and  $63.48^\circ$  correspond to (220), (311), (400), (333), and (440)

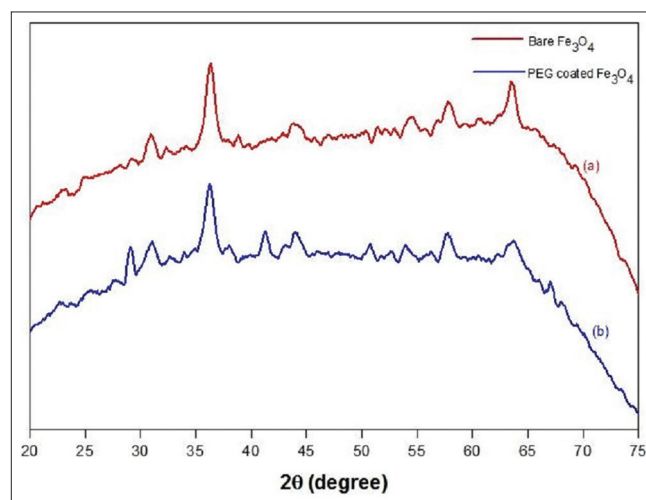
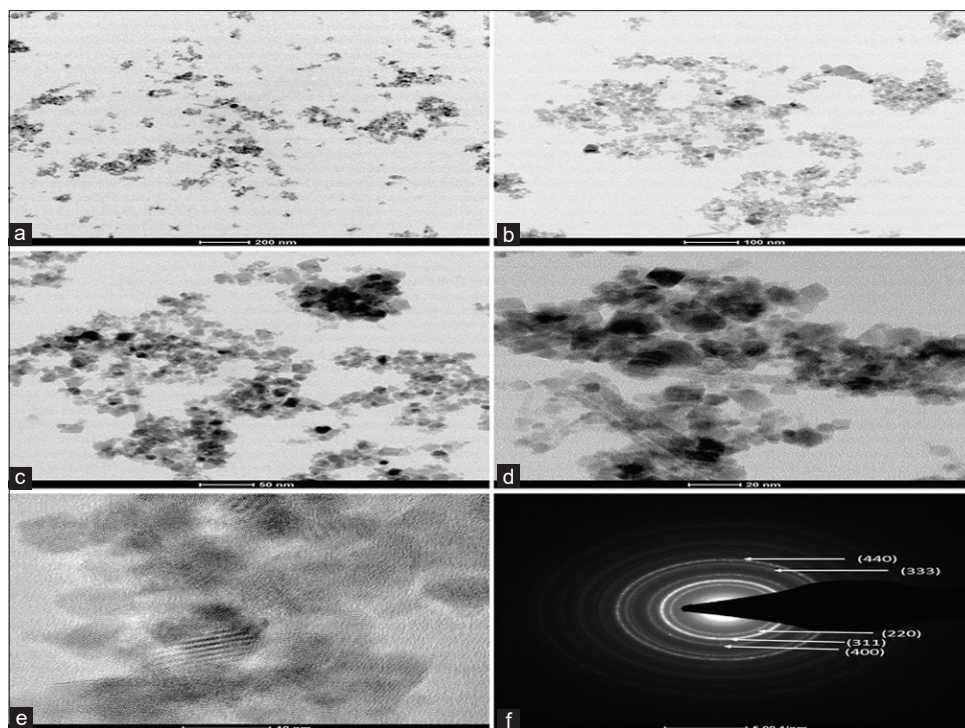
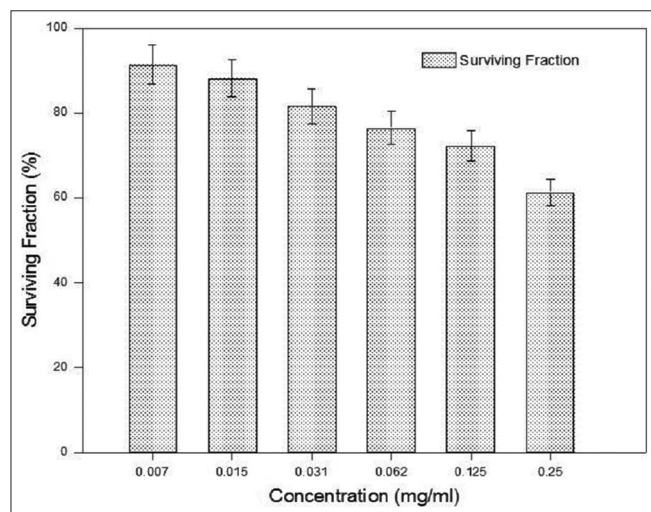


Figure 2: XRD patterns of (a) without polyethylene glycol coated  $\text{Fe}_3\text{O}_4$  and (b) with polyethylene glycol coated  $\text{Fe}_3\text{O}_4$



**Figure 3:** TEM micrographs (a-e) and (f) SAED pattern of polyethylene glycol coated Fe3O4



**Figure 4:** Percentage of HT-29 cell survival for different nanoparticle concentrations

planes, respectively, of inverse spinel magnetite phase. The XRD pattern reveals the formation of single phase with PEG-coated Fe<sub>3</sub>O<sub>4</sub> (b) and without (a) PEG-coated Fe<sub>3</sub>O<sub>4</sub> are in very close agreement with reported value of magnetite JCPDS Card No. 89-4319,  $a = 8.3952 \text{ \AA}$ . The crystalline size of with PEG coated Fe<sub>3</sub>O<sub>4</sub> [Figure 2b] and without [Figure 2a] PEG coated Fe<sub>3</sub>O<sub>4</sub> are estimated 9.85 nm and 10.00 nm, respectively. From X-ray line broadening, it is seen that coating reduces crystalline size of nanoparticles with PEG-coated Fe<sub>3</sub>O<sub>4</sub> [Figure 2b].<sup>[25]</sup> Figure 2 clearly shows that diffraction peaks for Figure 2a without PEG coated Fe<sub>3</sub>O<sub>4</sub> are stronger in intensity and narrower than Figure 2b with PEG

coated Fe<sub>3</sub>O<sub>4</sub>. It shows that crystallinity decreases for coated nanoparticles.<sup>[25]</sup>

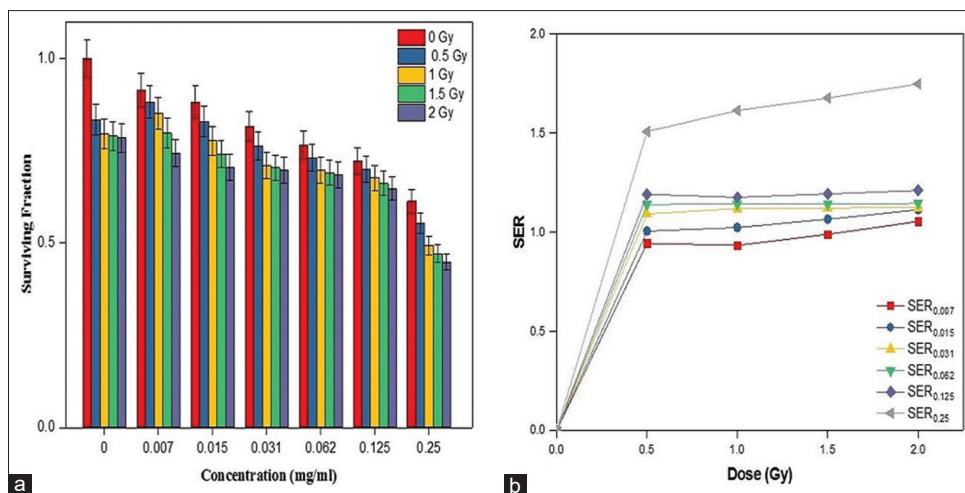
From TEM micrograph [Figure 3] of PEG-coated Fe<sub>3</sub>O<sub>4</sub> nanoparticle size calculated using image-j software and observed are in range of 9-20 nm in consistent with XRD result. TEM micrographs (a), (b), (c), (d), and (e) show nonagglomeration of PEG-coated Fe<sub>3</sub>O<sub>4</sub> nanoparticles from 200 to 10 nm magnifications. Shape of nanoparticles is polygonal and roughly spherical. The selected area electron diffraction pattern (SAED) of the nanoparticle is shown in Figure 3f. SAED pattern can be indexed to reflections of inverse spinel structure of Fe<sub>3</sub>O<sub>4</sub> and shows only diffraction intensity associated with highly crystalline Fe<sub>3</sub>O<sub>4</sub> which is in agreement with intensity of XRD peaks.

### Cytotoxicity evaluation of polyethylene glycol coated iron oxide nanoparticle

MTT assay done to evaluate cytotoxicity of PEG-coated Fe<sub>3</sub>O<sub>4</sub> nanoparticles for different concentrations such as 0.007, 0.015, 0.031, 0.062, 0.125, and 0.25 mg/ml without radiation exposure is shown in Figure 4. The percentage (%) of cell viability of HT 29 cancer cell incubated for 96 h with Fe<sub>3</sub>O<sub>4</sub> nanoparticles is 91.4%, 88.2%, 81.6%, 76.5%, 72.3%, and 61.3%, respectively. Result shows that as concentration increases cell killing increases. In this study, all the tested concentrations did not have considerable cytotoxicity.

### Radio sensitization enhancement by polyethylene glycol-coated iron oxide nanoparticle

HT-29 colorectal cancer cell lines were exposed with only radiation and radiation along with nanoparticles. Percentage



**Figure 5:** (a) Percentage of HT-29 cells survival for different concentration and at various doses of photon beam (b) Sensitization enhancement factor values of photon beam irradiations for different concentration and various doses

**Table 1: Sensitization enhancement ratio values for different concentration superparamagnetic iron oxide nanoparticles for various doses of radiations**

Dose (Gy)	SER <sub>0.007</sub>	SER <sub>0.015</sub>	SER <sub>0.031</sub>	SER <sub>0.062</sub>	SER <sub>0.125</sub>	SER <sub>0.25</sub>
0.5	0.94	1.00	1.09	1.14	1.19	1.50
1	0.93	1.02	1.11	1.14	1.17	1.61
1.5	0.98	1.06	1.12	1.14	1.19	1.67
2	1.05	1.11	1.12	1.14	1.21	1.74

SER: Sensitization enhancement ratio

cell viability observed for only radiation doses of 0.5, 1, 1.5, and 2 Gy is 83.4%, 79.6%, 79.0%, and 78.5%. Cell killing for only radiation observed is not significant ( $P > 0.05$ ). When radiation combined with higher nanoparticle concentration, i.e., of 0.25 mg/ml for doses 0.5, 1, 1.5, and 2 Gy, % cell viability observed to be 55.3%, 49.3%, 47.1%, and 44.9%, respectively, showing that when radiation combined with different nanoparticle concentration for doses 0.5, 1, 1.5 and 2 Gy, cell killing increased by 28.1%, 30.3%, 31.9%, and 33.6%, respectively.

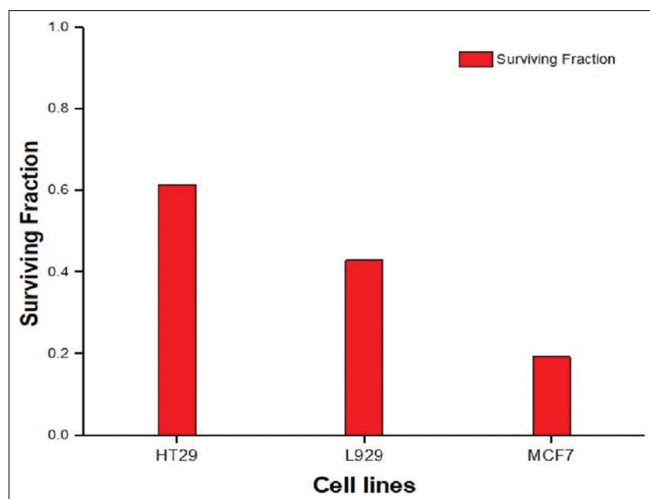
Dose response curves for different concentration are shown in Figure 5a. The percent cell viability fraction of HT-29 cells decreases with increasing concentrations of SPIONs as well as radiation doses. Sensitizer enhancement ratio (SER) values for 0.5, 1, 1.5, and 2 Gy were calculated by using the following formula  $SER_{xGy} = S.F_{xGy, Cont} / S.F_{xGy, NP}$  [26]. SER values for each dose for different concentrations are shown in Figure 5b. Greater SER value denotes radio sensitization by nanoparticle will be higher. SER values are increased from 0.94 to 1.74 for increased concentration from 0.007 to 0.25 mg/ml considering for all doses. Higher sensitization is observed for 0.25 mg/ml concentration of PEG coated  $Fe_3O_4$  nanoparticles. SER values are observed to be  $<1$  for doses 0.5, 1, and 1.5 Gy of the concentration 0.007 mg/ml [Table 1].

In summary, there is significant correlation in the percentage of the % cell viability observed for HT-29 cells incubated

with different concentration of SPIONs and SPIONs along with different radiation doses of 6 MVs photon beam with  $r^2$  value of 0.91 ( $P = 0.003$ ). Furthermore, statistically significant difference in the percentage of survival was observed between groups in which cells were exposed with only radiation doses and groups receiving radiation doses along with different SPIONs concentration with  $r^2$  value of 0.97 ( $P = 0.02$ ).

## DISCUSSION

Each substance used for cancer diagnosis or therapy is affected by biocompatibility and complicated metabolism. Therefore, the possibility of using one substance for different tasks (multi functionality) is particularly attractive.<sup>[21]</sup> Especially SPIONs have essential properties such as excellent biocompatibility, superparamagnetism,<sup>[19]</sup> physically and chemically stable, environmentally safety, ease of synthesis process, and surface treatment.<sup>[24]</sup> Our present results show that SPIONs can be used as radiotherapy sensitizer. One of the important steps toward the widespread usage of such nanoparticles is an assessment of the toxicity effect of these nanoparticles due to ROS production. Cytotoxicity has been assessed on different biological models using *in vitro* as well as *in vivo* studies. The *in vitro* studies are of more interest due to its simplicity, lower cost, and better control.<sup>[27]</sup> SPIONs have lower Z (26 vs. 79) and lower X-ray absorption enhancement factor (1.2 vs. 1.6, respectively) compared to



**Figure 6:** Comparison of cytotoxicity for different cell lines

gold nanoparticles at kV energy level according to Roeske's theoretical calculation.<sup>[28]</sup> SPIONs might still act as effective radiosensitizers at MV energies according to our results and others.<sup>[15,17,19,21,27,29]</sup>

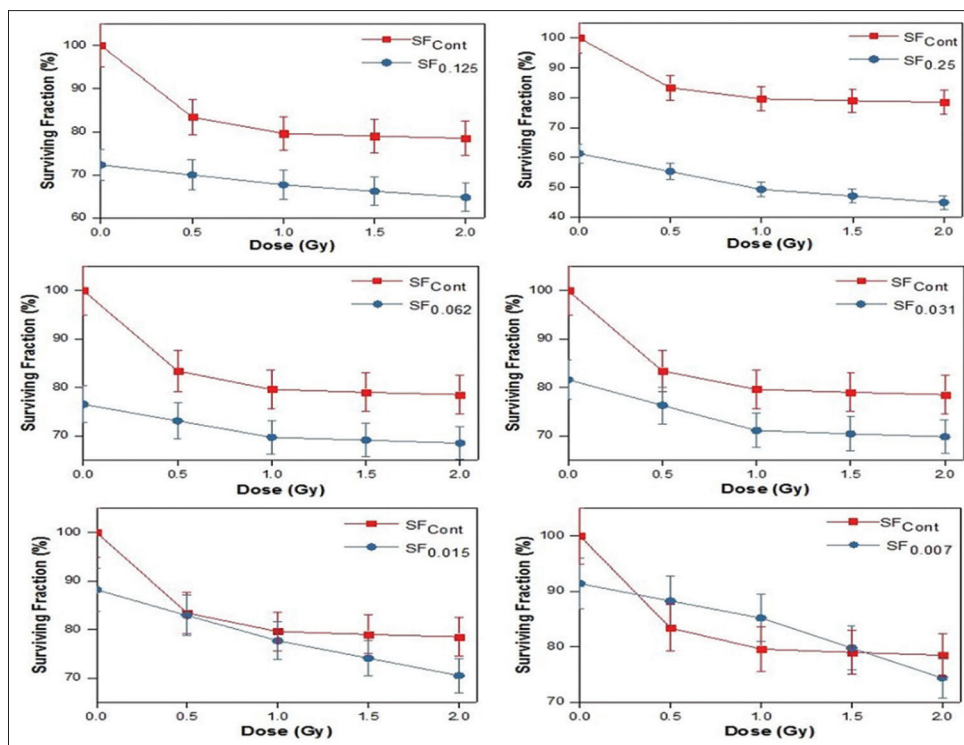
Huang *et al.*<sup>[21]</sup> estimated dose enhancement factor (DEF) in the case of cervical cancer cells (HeLa cell), is 1.6 at 1 Gy, 1.4 at 2 Gy, and 1.33 at 4 Gy and in the case of EMT cell, 1.6 at 1 Gy, 1.33 at 2 Gy, and 1.14 at 4 Gy. Here 0.040 mg/ml of cross-linked dextran-coated iron oxide (DCIO) showing greater cellular internalization and cytotoxicity compare to DCIO. DEF values found to be decreasing with increasing doses for same concentration.<sup>[21]</sup> *In vitro* study of SPIONs on human breast adenocarcinoma cell line (MCF-7), human mammary gland carcinoma cell line (MDAMB-231) and human ovarian carcinoma cell line (MDAH-2774) with radiation exposure of doses 0, 2, 4, 6, and 8 Gy at 6MV-energy carried out by Kirakli<sup>[15]</sup> for different concentrations such as 0.0125, 0.025, 0.05, 0.1, and 0.125 mg/ml, demonstrated that the highest radiosensitization were seen in MCF-7 and MDAH-2447 cells at 2 Gy (nanoparticle enhancement ratio [NER]:1.49 and 1.39, respectively), in MDA-MB-231 cells at 4 Gy (NER: 1.20). NER values decrease with increasing doses. Khoei *et al.*<sup>[30]</sup> studied sensitization effect of NH<sub>2</sub>-NanoMag (iron oxide nanoparticle with amino-group dextran coating) using MV photons produced by a LINAC (linear accelerator) on human prostate cancer DU145 cells. Obtained DEF values are 1, 1.21, 1.24, 1.22, and 1.21 for radiation doses 0, 1, 2, 4, and 6 Gy. In this study, 1, 2, and 3 mg/ml concentrations were used and cell viability found to be decreasing with concentrations also cell survival fraction decreases with the increase of radiation dose for 1 mg/ml NH<sub>2</sub>-NanoMag. Razaei *et al.*<sup>[27]</sup> carried out the cytotoxicity evaluation of dextran-coated iron oxide nanoparticles (IONPs) at different concentrations (0.010, 0.040, and 0.080 mg/ml) on HeLa and MCF-7 cell lines.

The radiosensitivity effect was evaluated for the nanoparticles which were incubated with the cells at different concentrations for 24 h and afterward irradiated with different doses (0, 2, 4, 6, and 8 Gy) of 6 and 12 MeV electron beams. Toxicity results of the nanoparticles at 0.010 and 0.040 mg/ml concentrations showed no significant cytotoxicity effect. The cells survival rates in groups receiving radiation in the absence and presence of IONPs showed a significant difference. The radio sensitivity enhancement induced by the nanoparticles in MCF-7 cell line was more than it in HeLa cell line. The average of radiosensitization enhancement factor at 0.01, 0.04, and 0.08 mg/ml concentrations and under 6 MV irradiations obtained as 1.13, 1.19, and 1.25, and 1.26, 1.28, 1.29 for HeLa, and MCF-7 cells, respectively. For 12 MVs electron beam, the values of 1.17, 1.26, and 1.32, and 1.29, 1.32, and 1.35 were obtained for the cells at the mentioned concentrations, respectively. The significant differences were observed in radio sensitization enhancement between 6 and 12 MeV electron beams irradiations.

Compare to these studies, we found greater radio sensitization by PEG coated SPIONs for concentration 0.25 mg/ml at 2 Gy dose of 6 MVs photon beam (SER: 1.74). We have studied biocompatibility of PEG-coated SPIONs on normal cell line, i.e., L929, results shows about 57% cell killing for 0.25 mg/ml concentration<sup>[7]</sup> [Figure 6]. As we increase concentration above 0.25 mg/ml, normal cell killing also increases. So it might be useful to take concentrations up to 0.25 mg/ml. Furthermore, there has been no study found that SPIONs treated on colorectal cancer cell lines (HT-29). In our study, we found that radiosensitization increases with concentration of nanoparticles which is consistent with other studies. We compared surviving fraction of cell lines exposed to radiation and nanoparticles with control (i.e, only radiation) for all concentrations[Figure 7]. It has been seen that HT-29 cell survival reduced up to minimum 20% when it is exposed with radiation doses for concentrations 0.031, 0.062, 0.125, and 0.25 mg/ml compare to radiation doses alone. Figure 7 explains how cell survival effectively reduced when HT-29 exposed to only radiation doses and to radiation doses along with PEG coated SPION's for each concentrations of 0.031, 0.062, 0.125, and 0.25 mg/ml, but for lower two concentrations survival values observed to be nearly same for radiation alone and radiation along with SPIONs. Hence, 0.031, 0.062, 0.125, and 0.25 mg/ml might be applicable to work as radiotherapy sensitizer in colorectal cancer.

SER values calculated for each doses and concentrations are tabulated, as shown in Table 1. As doses increase, there is a slight increment observed in SER values for concentrations 0.007, 0.015, 0.031, 0.062, and 0.125 mg/ml but for 0.25 mg/ml concentration of nanoparticle, SER values increase effectively with doses [Table 1].

Fe<sub>3</sub>O<sub>4</sub> was found to be cytotoxic as it contains Fe<sup>2+</sup> ions and helps in the formation of ROS which leads to cell death through



**Figure 7:** Comparison of surviving fraction of cells exposed to radiation dose alone (Control) with radiation dose exposed with superparamagnetic iron oxide nanoparticles concentrations

damage of mitochondria.<sup>[19]</sup> The pathway of production of ROS is the Haber-Weiss reaction which results in the generation of the highly reactive hydroxyl radical from the reaction between super oxide and hydrogen peroxide.<sup>[19,29]</sup> Radiation therapy promotes the production of superoxide anion through leakage of electrons from the electron transport chain. Iron oxide nanoparticles can then catalyze the reaction to produce highly ROS.<sup>[29]</sup> This is the important parameter in the use of SPIONs as a radiotherapy sensitizer. Cytotoxicity of nanoparticles is the combined effect of ROS production by radiation and SPIONs. It depends on the several factors such as size, concentration, incubation time of nanoparticles, cell line, amount of radiation doses, energy, and type of radiation.<sup>[27]</sup> In above cases as concentration of nanoparticle, amount of radiation doses delivered and energy of radiation varies, sensitization effect on cell lines also changes.<sup>[21,27,29,30]</sup> In our research, we observed for 0.25 mg/ml concentration MCF-7 cell line shows 80.7% cell killing while for normal (L929) and HT-29 cell line it's been 57% and 38.7%, respectively.<sup>[17]</sup> We found that MCF-7 shows more sensitivity to SPIONs itself compare to normal (L929) as well as HT-29 cell line, as shown in Figure 6. Cytotoxicity observed for HT-29 cells is less when it exposed to only radiation or only nanoparticles. Hence, there is a need to increase cytotoxicity of HT-29 by combining SPIONs and radiation together to achieve better tumor control.

On the other hand, as iron is an essential factor for cell growth and its multiplication in view of its role in the activity of DNA synthesis and for the reduction of ribonucleotides to deoxy ribonucleotides. It needs a continuous supply of iron to

maintain activity. Thus, the essentiality of this metal together with its potential toxicity suggests that cellular iron metabolism needs to be highly regulated.<sup>[31]</sup> In our study, we found that cells exposed to SPIONs of concentrations 0.007 and 0.014 mg/ml for 0.5, 1, 1.5, and 2 Gy have shown slightly greater survival compared to cells exposed with only radiation doses, as shown in Figure 5a which is controversial. This suggests that these two concentrations promote cell growth along with radiation and effective cell killing is observed for concentration onward 0.031 mg/ml up to 0.25 mg/ml suggesting that these concentrations may be applicable for radiotherapy to work as sensitizer.

Various studies revealed that within a 1-100 nm range, 50 nm NPs show maximum cellular uptake, with 14-20 nm NPs having a higher endocytotic rate than the 100 nm NPs.<sup>[32]</sup> In addition, coated NPs (surface charged) display internalization more readily than their plain counterparts because of increased surface potential resulting in higher affinity for cells.<sup>[15]</sup> Synthesized PEG-coated Fe<sub>3</sub>O<sub>4</sub> nanoparticles are biocompatible, stable, super paramagnetic, and of size in range of 10–20 nm.<sup>[17]</sup> In summary, PEG-coated Fe<sub>3</sub>O<sub>4</sub> nanoparticles are well suited to work as radiotherapy sensitizer in colorectal cancer. Side effects on normal tissue due to incremented doses to achieve better TCP can be reduced using sensitization effect of nanoparticles.

## CONCLUSIONS

These results reveal that PEG coated superparamagnetic iron oxide nanoparticles have synergetic effect on HT-29 cell

lines while it used along with radiation doses of MV energy X-rays. Greater SER values obtained while using SPIONs as a radiosensitizer even it has less atomic number. In case of colorectal cancer, local tumor control (TCP) is achieved at a minimum risk of normal tissue complications (normal tissue complication probability, NTCP) by using SPIONs.

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### Conflicts of interest

There are no conflicts of interest.

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