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Application of PEGylated cobalt and nickel ferrite nanoparticles as radiosensitizing agents for breast cancer cells

Daniele Alves Fagundes[1], Joice Yoko D'Alessandro Idehara[2], Nayara de Almeida Gonzaga[3], LiliamViana Leonel[4], Raquel Gouvêa dos Santos[5], and José Domingos Ardisson[6]

[1]*daniele.fagundes@cdtn.br, Centro de Desenvolvimento da Tecnologia Nuclear (Belo Horizonte - MG, Brazil)*
[2]*joice.idehara@cdtn.br, Centro de Desenvolvimento da Tecnologia Nuclear (Belo Horizonte - MG, Brazil)*
[3]*nayara.gonzaga@cdtn.br, Centro de Desenvolvimento da Tecnologia Nuclear (Belo Horizonte - MG, Brazil)*
[4]*lvl@cdtn.br, Centro de Desenvolvimento da Tecnologia Nuclear (Belo Horizonte - MG, Brazil)*
[5]*santosr@cdtn.br, Centro de Desenvolvimento da Tecnologia Nuclear (Belo Horizonte - MG, Brazil)*
[6]*jdr@cdtn.br, Centro de Desenvolvimento da Tecnologia Nuclear (Belo Horizonte - MG, Brazil)*

1. Introduction

Breast cancer is one of the most common malignancies affecting women worldwide. Radiotherapy is a critical treatment option against these locally advanced tumors [1]. However, due to tumor radioresistance and normal tissue radiosensitivity, the major challenge of this treatment is to give tumor cells a lethal dose of radiation, sparing surrounding healthy cells [2,3].

Radiosensitizers are physicochemical agents given in conjunction with radiation to potentiate DNA damage [4,5]. In recent years, there has been a considerable interest in the utilization of heavy metal-containing nanoparticles (NPs) due to their higher energy absorption coefficient compared to that of water or soft tissues and enhanced permeability and retention (EPR) effect. When exposed to electromagnetic radiation, heavy metal NPs may emit secondary and Auger electrons increasing the therapeutic effect [2,3].

Among the NPs, cobalt and nickel ferrite NPs stands out for their chemical stability, low cost and high resistivity [6]. Moreover, the iron present in the ferrite structure can catalyze Haber-Weiss and Fenton reactions and, consequently, increase the efficiency of radiotherapy through the production of reactive oxygen species (ROS) [2,3].

For use of NPs in the biomedical area, factors such as particle size, narrow size distribution and possibility of surface functionalization with different binders are significantly relevant [7,8]. To maintain the stability of NPs in colloidal suspensions and to promote biocompatibility, functionalization strategies are indispensable [8]. To prevent opsonization, one of the most promising strategies is the functionalization with polyethylene glycol (PEG), a non-immunogenic, hydrophilic and non-toxic molecule, approved for human internal use by the Food and Drug Administration (FDA). PEG molecules can be covalently bonded or physically adsorbed on the surface of the particles allowing a longer body circulation time [9].

In this context, we investigated the biocompatibility and radiosensitizing potential of PEGylated cobalt and nickel ferrite nanoparticles.

2. Methodology

Citrate-coated cobalt and nickel ferrite nanoparticles were synthesized by the polyol method using an *ex-situ* (two-step) route, according to the procedure described by LEONEL et al. 2018 [10]. Posteriorly, the NPs were functionalized with polyethylene glycol (PEG) via carbodiimide chemistry [11,12].

Normal human fibroblast (MRC-5) and breast cancer (MCF-7) cells were used for the biological assays. The *in vitro* evaluations were performed using the contrast phase microscopy to evaluate cell structural and morphological alterations and the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay to investigate metabolic cell viability. The cells were irradiated by a MDS Nordion multipurpose panoramic irradiator equipped with a dry-stored ^{60}Co source (2,200 TBq). Gamma radiation doses of 1 and 3 Gy (57 - 60 Gy.h^{-1}) were used.

3. Results and Discussion

Cobalt and nickel ferrite nanoparticles with nearly spherical morphology and low aggregation degree (Figure 1a), mean diameter of 4.9 ± 0.1 nm (PEG-CF) and 5.3 ± 0.1 nm (PEG-NF), narrow size distribution (Figure 1b) and hydrodynamic size of 53.7 ± 0.5 nm (PEG-CF) and 33.8 ± 0.2 (PEG-NF) were obtained.

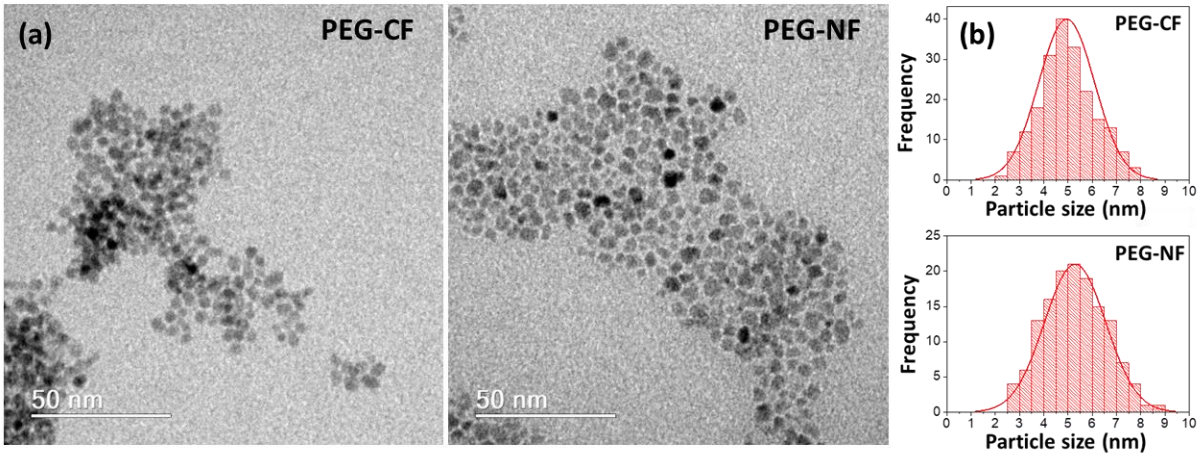

Figure 1: (a) Transmission electron microscopy images and (b) particle size distributions of PEG-NPs.

MRC-5 lineage was used as a model of non-tumor cells for PEG-NPs biocompatibility assessment. Cells exposed to different concentrations of PEG-NPs presented similar morphology and density compared with the control samples (Figure 2a) suggesting NP biocompatibility. Indeed, PEG-NPs were biocompatible up to 100 μg.mL^{-1}, with metabolic viability around 80 - 90 % of the control (Figure 2b). On the other hand, PEG-NPs concentrations higher than 100 μg.mL^{-1} showed cytotoxic effects, reaching approximately 55 % of metabolic cell viability at 500 μg.mL^{-1}.

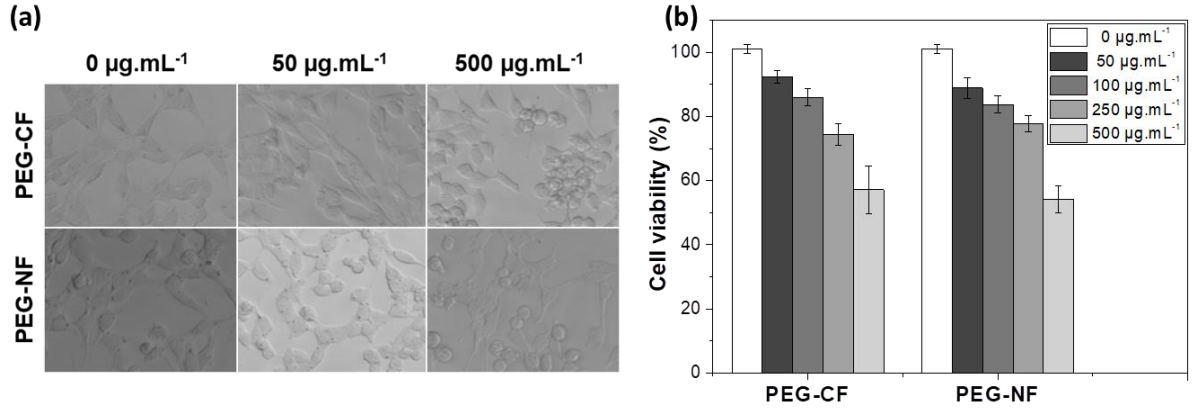

Figure 2: (a) Photomicrographs (400x magnification) and (b) metabolic viability of MRC-5 cells after 24 h treatment with different concentrations of PEG-NPs.

Unlike the effect on MRC-5 cells, PEG-NPs induced about 35 % cell death for the MCF-7 breast cancer cells at the concentration of 100 µg.mL^{-1}, reaching approximately 60 % at 500 µg.mL^{-1} concentration (Figure 3a), suggesting selectivity for tumor cells compared to the normal cells studied.

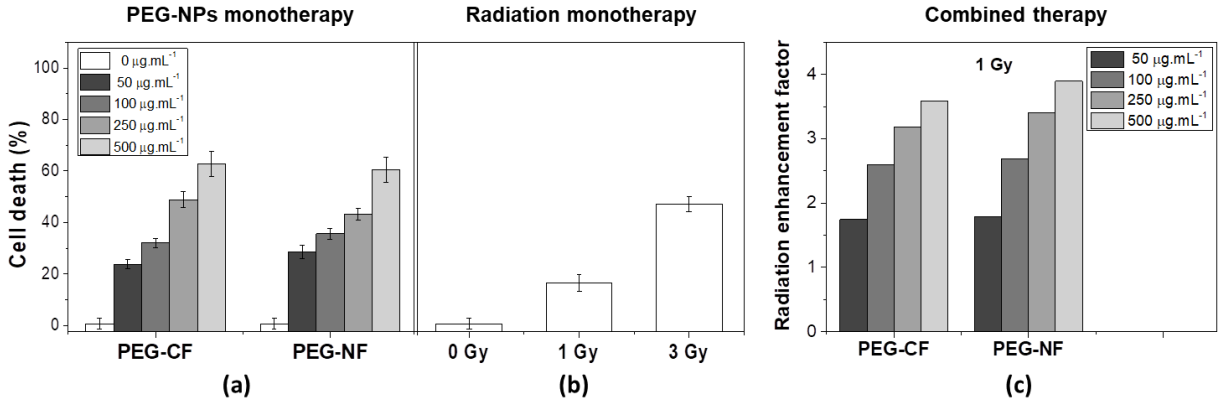

Figure 3: Effect induced on MCF-7 cells after 24 h of (a) PEG-NPs monotherapy, (b) gamma radiation monotherapy and (c) combined therapy.

To investigate the radiosensitizer potential of PEG-NPs, the effect of gamma radiation monotherapy was evaluated. According to Figure 3b, it was possible to observe that gamma radiation reduced the metabolic viability of cells, reaching 17 ± 3% and 47 ± 3% of MCF-7 cell death at 1 and 3 Gy doses, respectively. The combination of PEG-NPs with gamma radiation (Figure 3c) resulted in up to four-fold increase in the radiation therapeutic efficacy against breast cancer cells, indicating the radiosensitizing effect of PEG-NPs. This increase in the combined therapy cytotoxic response can be attributed to the capacity of the ferrite NPs to absorb ionizing radiation, emit secondary electrons and act as a catalyst for Haber-Weiss and Fenton reactions, which consequently may induce direct and indirect DNA damage [2,3,5]. More studies are needed to determine the metabolic pathways involved in the radiosensitizer effect of PEG-NPs.

4. Conclusions

PEGylated cobalt and nickel ferrite nanoparticles (PEG-NPs) with suitable characteristics for biomedical application such as reduced size, narrow size distribution, low aggregation degree, hydrophilicity and high crystallinity were obtained. PEG-NPs proved to be biocompatible up to 100 µg.mL^{-1} concentration. Gamma

radiation monotherapy at 1 Gy dose showed only slight effects on MCF-7 breast cancer cells metabolism. However, combination of PEG-NPs with 1 Gy gamma radiation resulted in up to four-fold increase in the radiation therapeutic efficacy. These results indicate that PEG-NPs are suitable candidates for application as radiosensitizing agents for breast cancer cells.

Acknowledgements

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