[801] **EVALUATION OF LOW DOSES OF GAMMA IRRADIATION IN THE FORMATION OF MINERALIZATION NODULES IN OSTEOBLASTS CULTURE**

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Introduction: Osteoblasts are specialized fibroblasts that secrete and mineralize the bone matrix. The mineralized extracellular matrix is mainly composed of type I collagen, osteocalcin, and the inorganic mineral hydroxylapatite1. The use of radiation as therapy in some cancers causes great bone loss. However, low dose radiation may have the opposite effect. Low dose X-irradiation on osteoblastic culture had effects on proliferation and differentiation with increase of mineralization nodules2. However, there is little information on the potential therapeutic efficacy of low-dose gamma-irradiation in the formation of mineralization nodules.
Objective: To evaluate the effects of irradiation with 60Co γ-rays in low doses in the formation of mineralization nodules in culture of osteoblasts.

Methods: MC3T3-E1 cells were bought by the Banco de Células do Rio de Janeiro, Brazil (MC3T3-E1 Subclone 14). The cells were cultured in α-MEM medium consisting of 10% FBS and without β-glycerophosphate and L-ascorbic acid (GIBCO, Custom Product, Catalog No. A1049001) (Zhao Y, Guan H, Liu S et al. Biol. Pharm. Bull. 2005, 28(8):1371-1376).
Plating efficiency assays: cells were plated at a density of 100 cell/plate into 60 mm Petri dishes. After 14 days the places were stained with violet crystal and the colonies were counted.
Mineralization assays: cells were cultured in the same medium with 10 mM □-glycerophosphate and 50 mg/ml ascorbic acid, and analyzed on days 7, 14 and 21. Osteoblast culture irradiation assay: cells were plated at a density of 1x 105 cells/plate on 60 mm dishes and the next day were irradiated by 60Co source with 0 (as the control), 0.5, 1.0, 1.5 and 2.0 Gy using the GammaCell 220 – Irradiation Unit of Canadian-Atomic Energy Commission Ltd. (CTR-IPEN). On day 21 of culture, undifferentiated (without ascorbic acid and β-glycerophosphate), differentiating cells (0 Gy) and irradiated cells at different doses, the medium was removed, cells were washed with phosphate buffer saline, fixed with 70% ethyl alcohol and stained with Alizarin red S (Sigma). All in three biological replicates (a total of 54 samples) and multiple comparisons were assessed by One-way ANOVA followed by Bonferroni´s tests with GraphPad Prism version 6.0 software. $P < 0.05$ was considered statistically significant.
Results: Plating efficiency (PF) analysis is generally considered to be the gold standard of assays for testing the sensitivity of cell lines to ionizing radiation or other cytotoxic agents in vitro. The results obtained were a PF of 30% for non-irradiated culture, however, the irradiated culture obtained 40% in relation to the no-irradiated one, already with 0.5 Gy, and this percentage was maintained in the other larger doses. Regarding the evaluation of the formation of mineralization nodules, significant difference in 0.5 Gy group was observed compared with the control group (0 Gy), 64.7±1.8 and 53.0±0.9, respectively. The groups of 1.0, 1.5 and 2.0 Gy obtained a decrease in the mineralization nodules. The data obtained with increasing irradiation produced an increase of mineralization nodules up to 0.5 Gy and in the higher doses had a decrease. Applying the data in a non-linear function it is observed that the line has a decreasing tendency with the negative angular coefficient. This analysis is in agreement with the hormesis model, in which low doses induce a stimulatory effect while high doses cause inhibition4.
Conclusions: This study is one among the first that investigating the biophysics of low-dose gamma-irradiation on MC3T3-E1 culture, focusing on the potential applications in bone replacement therapy.
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Keywords: osteoblast, gamma irradiation, mineralization