Cytogenetic analysis of ^{60}Co γ-radiation-induced chromosome damage and simulations using the Geant4 Monte Carlo toolkit

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Abstract. The study involves the determination and quantization of radiation damage on a cellular level and the replication of this energy deposition using the Geant4 Monte Carlo toolkit, developed for particle transportation simulations at CERN. The detection of micronuclei in binucleated cells was used to analyse the effects of the radiation. Results from micronucleus assays of rat brain endothelial and Chinese hamster ovary cells that have been irradiated with gamma rays, produced by a Co-60 teletherapy unit at iThemba LABS, are presented. Linear-quadratic dose response curves were determined for both cell lines. The simulated results of the Co-60 setup, irradiation and other relevant features offered by the Geant4 toolkit are also discussed. Finally, prospective microdosimetric studies and simulations of damage caused by DNA-incorporated I-123 will be mentioned.

1. Introduction

A standard method used to determine the combined effects of ionizing radiation-induced damage and repair is cytogenetic analysis (or analysis of chromosomes). Chromosomal aberrations resulting from non- or misrepaired radiation-induced double strand breaks can be detected using this method.

The cytokinesis-block micronucleus (CBMN) cytome assay is one such cytogenetic method [1]. After *in vitro* irradiation, cells are blocked from performing cytokinesis (the division of cytoplasm and cell membrane) by the addition of cytochalasin-B (cyt-B). Cyt-B is an inhibitor of actin polymerization required for the formation of the microfilament ring that constricts the cytoplasm between the daughter nuclei during cytokinesis [2]. Since nuclei can still divide, after one division, cells will consequently appear as binucleated (BN) cells. DNA damage events are scored specifically in once-divided BN cells and include micronuclei (MNi).

MNi, possibly resultant from the radiation damage, are small extranuclear bodies detected around these BN cells. MNi are formed from acentric chromosome fragments and whole chromosomes that lag behind in anaphase and are left outside the daughter nuclei in telophase [3]. Additionally, ionizing radiations are more effective on cells which have a greater reproductive activity [4]. Therefore MNi provide a convenient and reliable index of both chromosome breakage and loss; and as the number of radiation-induced MNi is strongly correlated with radiation dose and quality, the CBMN assay is an appropriate test for biological dosimetry and the assessment of intrinsic radiosensitivity [5], [6]. Although the CBMN assay is generally applied on lymphocytes, micronuclei can be detected in all types of cells, as long as they are capable of division.

The knowledge of absorbed dose is required for evaluation of observed radiobiological effects and to predict or compare the effectiveness of different radiations. Due to small cellular dimensions direct dose measurement is virtually impossible [7]. According to the stochastic character of all interaction processes, a large number of pathways exist by which energy may be deposited. Because these energy exchanges are random processes, the Monte Carlo technique is an appropriate tool for their simulation; as well as a feasible method to obtain correct dose values under considerations of all irradiation aspects like geometry and activity distributions [8].

Geant4 is an open source Monte Carlo toolkit, based on object orientated programming rules using the C++ language, which provides functions for simulating the passage and interactions of particles through matter [9]. The main advantage of this toolkit is its openness to modification and extensions. The code is freely downloadable from the Geant4 web site as well as detailed descriptions of the toolkit design and the physical fundamentals may be found in the "Geant4 User's Guide for Application Developers" and the "Physics Reference Manual" [10].

In this study, CBMN assays were used to analyze the effects of γ-radiation from a ^{60}Co source on rat brain endothelial (bEND5) and Chinese hamster ovary (CHO-K1) cells. Dose-response curves were then determined for both cell lines. These linear-quadratic equations will be used as reference curves in future work concerning the relative biological effectiveness of Auger electrons from DNA-incorporated ^{123}I.

Geant4 was used to simulate the experimental setup and the decay of a ^{60}Co source and to determine the energy deposition in a certain volume. From these simulations, an equation relating activity to dose rate was determined. The predicted dose rate was then compared to the measured value. This basic simulation was done to determine the accuracy and reliability of Geant4 for future work regarding the simulation of energy depositions due to Auger electrons from the full decay of ^{123}I (incorporated into the anatomy of a cell).

2. Experimental procedure

2.1. Biological experiments

2.1.1. Cell preparation and irradiation

The samples were individually irradiated using a ^{60}Co teletherapy unit (Eldorado 76, Atomic Energy of Canada Ltd.) at iThemba LABS-NRF, Somerset West. The irradiation setup consisted of a field size of 30 × 30 cm^2 and a source-to-surface distance (SSD) of 75.6 *cm*. A 6 *mm* thick Perspex sheet was placed at 75 *cm* to act as build-up material and insure electronic equilibrium. A 4.9 *cm* slab of Perspex was placed 3.5 *cm* from the build-up to aid in dose deposition due to backscatter. On 5 June 2013 the dose rate of the ^{60}Co unit was 0.49 *Gy/min*, measured according to the International Commission on Radiation Units and Measurements protocol. According to accompanying documentation, the teletherapy unit had an activity of 48.4 *TBq* on that date.

The bEND5 cells were allowed to grow to confluence; incubated at 37°C in a humidified atmosphere of CO_2. On the day of irradiation, the cells were trypsinized from the culture flasks. A hemocytometer was used to obtain a cell count of approximately 3×10^5 cells/*ml*. Cell culture medium (RPMI 1640) was added to the cell mixture as needed in order to obtain the required concentration. Tubes were labeled according to the planned exposure values (namely 0 – 4 *Gy* in 0.5 *Gy* increments, hence 9 dose values). Into each of these tubes, concentrated cell mixture and medium were added. The tubes were then individually irradiated using the ^{60}Co unit. After the irradiation, the contents of each tube were divided into 2 wells of a 6-well plate, labeled A and B with their corresponding dose value, culminating in 18 separate samples. To block cytokinesis, 3.76 *μl* of Cyt-B was added to each well. The cells were then stored in an incubator at 37°C overnight (approximately 20 – 24 hours). After incubation, the cells were harvested by trypsinization, treated with a hypotonic solution of 75 *mM* KCl, fixed once with a 4/1/5 methanol/acetic acid/Ringer's solution mixture and stored overnight at

4°C. On the day of slide preparation, the cells were fixed three times with a 4/1 methanol/acetic acid as described previously by Vandersickel, et al. [11]

In a similar procedure, the CHO-K1 cells were harvested after incubation by trypsinazation. A cell count of approximately 1.5×10^5 cells/*ml* was obtained. Nine small Petri dishes (35 *mm*) were labeled according to the exposed dose; each containing a 22 *mm* glass cover slip. Into each of these dishes, 0.2 *ml* of CHO-K1 cells and medium were added, and then individually irradiated. Similarly, Cyt-B was added after irradiation, and the cells were incubated for approximately 24 hours. Afterwards, the supernatants were removed, the seeded cells treated with a hypotonic solution of 75 *mM* KCl and fixed once with a 1/3 methanol/acetic acid solution. Supernatants were then removed; the cover slips were allowed to air dry and then stored at room temperature until slide preparation.

2.1.2. Slide preparation and MN scoring

Duplicate slides were prepared for each bEND5 cell culture. The supernatants of each tube was removed and retained for storage. Forty *μl* of the cells were dropped on each of the 2 clean slides and air dried. The supernatants were replaced with the remaining cells in the tube for refrigerated storage. Finally, the slides have labels A1, A2, B1, and B2 for each exposure value, resulting in 36 slides in total. The resulting DNA damage due to the radiation was quantified by numerating the number of MNi per BN cell. Two drops of DAPI Vectashield were added to each slide to stain the nuclei. The slides were then each covered with a cover slip and allowed to dry. A semi-automatic counting method was implemented using the Msearch Module of the Metafer 4 automated imaging system [12]. This was done for all 36 slides. To analyse the obtained results, the number of MNi per BN cell was averaged over the 4 samples (A1, A2, B1, and B2) of each exposure value and then normalized to per 1000 BN cells. This value was then plotted against the dose and a linear-quadratic curve was fitted to the data points (using the MATLAB v 7.6 analysis package). Once the intrinsic MNi of the cell culture has been subtracted (obtained from the 0 *Gy* sample), the curve has the form:

$$y = \alpha \cdot D + \beta \cdot D^2$$

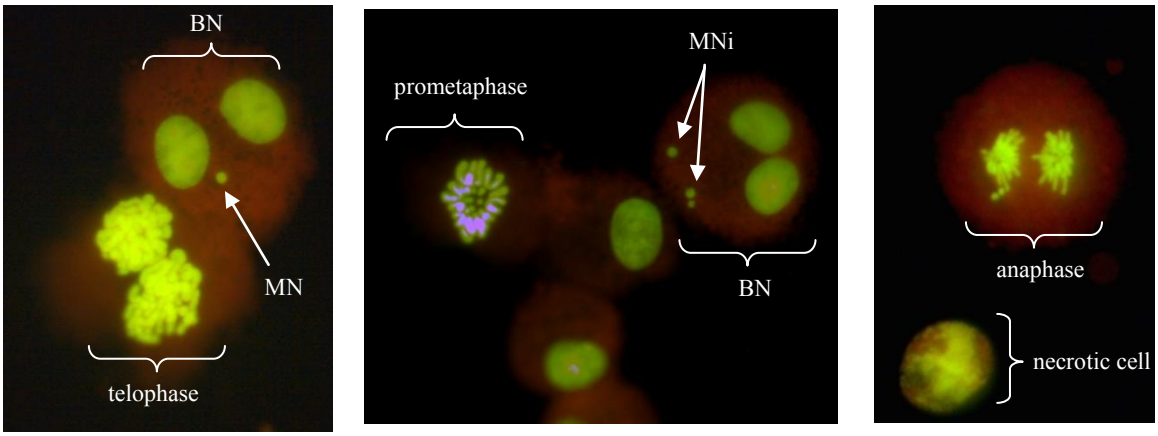

Figure 1. CHO-K1 stained with Acridine Orange. The cytoplasm emits an orange/red colour whereas the nuclei are a yellow / bright green colour. Pictured are some cells in varying phases of mitosis.

A working solution of Acridine Orange stain was made by adding 0.5 *ml* Acridine Orange solution to 45 *ml* buffer solution (pH 6.8). For each of the CHO-K1 cover slips: the cover slip was immersed in the working solution of Acridine Orange for 1 min. The cover slip was then rinsed with distilled water. Finally, the cover slip was immersed in the buffer solution (pH 6.8) for 1 min to de-stain. A drop of buffer solution was placed on a clean microscope slide and the cover slip was carefully mounted upside-down, avoiding any bubble formation. The edges of the cover slip were sealed with Cutex to prevent the drying of the slide. The MNi per BN cell were then counted, using ZEISS Axio Scope A1 and FITC filter (see figure 1), up to 200 cells. This was done for each of the 9 dose points. As

previously, the number of MNi per BN cell was normalized to per 1000 BN cells; plotted against the exposed dose and had a linear-quadratic curve fit to the data points.

2.2. Monte Carlo simulations

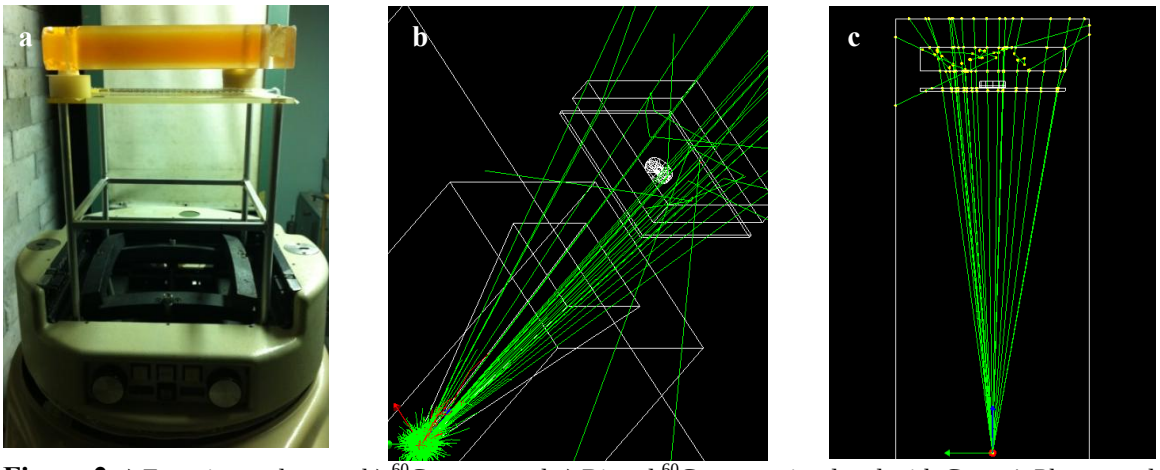

Figure 2.a) Experimental setup. b) ^{60}Co setup and c) Biased ^{60}Co setup simulated with Geant4. Photon and electron tracks are shown in green and red, respectively.

Geant4.9.6 and Microsoft Visual C++ 2010 Express was used on a Windows 7, Intel Core2 Duo @ 2x2 GHz, 3 GB RAM platform to reproduce the experimental setup. In a world geometry filled with air at standard temperature and pressure, a Perspex build-up was placed at 75 *cm* from the source, and a slab of Perspex was placed 79.1 *cm* from the source to act as backscatter material. The source was placed at the origin and a volume of water (r = 2.65 *cm*, h = 4 *mm*, ρ = 1 g/cm^3) was placed at 75.6 *cm*. A certain activity (*Bq* = disintegrations/sec) of source material was simulated and the energy deposited per second, in the investigated volume, was measured. This energy deposition rate was then converted to an absorbed dose (*Gy* = *J/kg*) rate. Two different simulations were constructed: a full, isotropic decay of a ^{60}Co source, and an energy- and directionally biased simulation. In both the RanecuEngine randomizing engine was used [13]. The experimental and simulated setups can be seen in figure 2. Simulations were run up to 10 *MBq*.

In the "^{60}Co setup", a ^{224}U housing with a 10x10 cm^2 square-aperture was placed around the origin, to produce a 30x30 cm^2 collimated field at 75 cm. In this simulation, the ^{60}Co nuclei were allowed to isotropically β^- -decay to their ^{60}Ni ground state, producing all of the documented particles and their spectrum of energies. In the physics list, the interaction processes were individually set for each type of particle (e.g. gammas can undergo the Photoelectric effect, Compton scattering, gamma conversion leading to pair-production).

In the "Biased ^{60}Co setup", the production of a 1.173 and 1.332 MeV pair of γ-rays was considered as one decay. These photons were produced within an 11.2° angle in the forward direction. The built-in QBBC reference physics list was used to govern the particle interaction processes in this simulation [14].

3. Results and Discussion

3.1. Biological results

From figure 3 we can see that the bEND5 cells are vastly more radiosensitive than the CHO-K1 cells, also indicated by the larger α-value in table 1. For bEND5 cells we see that points above 2.5 *Gy* reach a plateau, indicating that the cells have been saturated by the radiation. The limited number of cells on each slide also limit the number of MNi/BN cells. The linear-quadratic trend is present in both cell lines, as it should. The values for each dose point will differ when compared to values available in literature, as they are dependent on the specific cell line and the scoring method used [15].

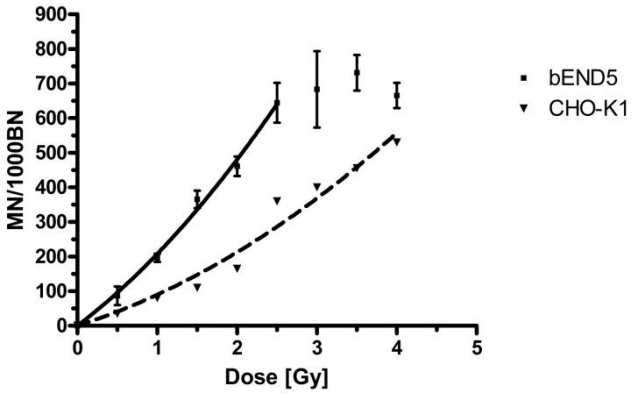

Figure 3. Number of micronuclei per 1000 bi-nucleated cells versus dose and the resulting dose-response curve.

Table 1. ^{60}Co radiation dose response parameters for MN frequencies.

Cell line	α (Gy^{-1})	β (Gy^{-2})
bEND5	175.0 ± 31.8	32.5 ± 15.1
CHO-K1	73.2 ± 22.3	16.48 ± 6.8

3.2. Monte Carlo results

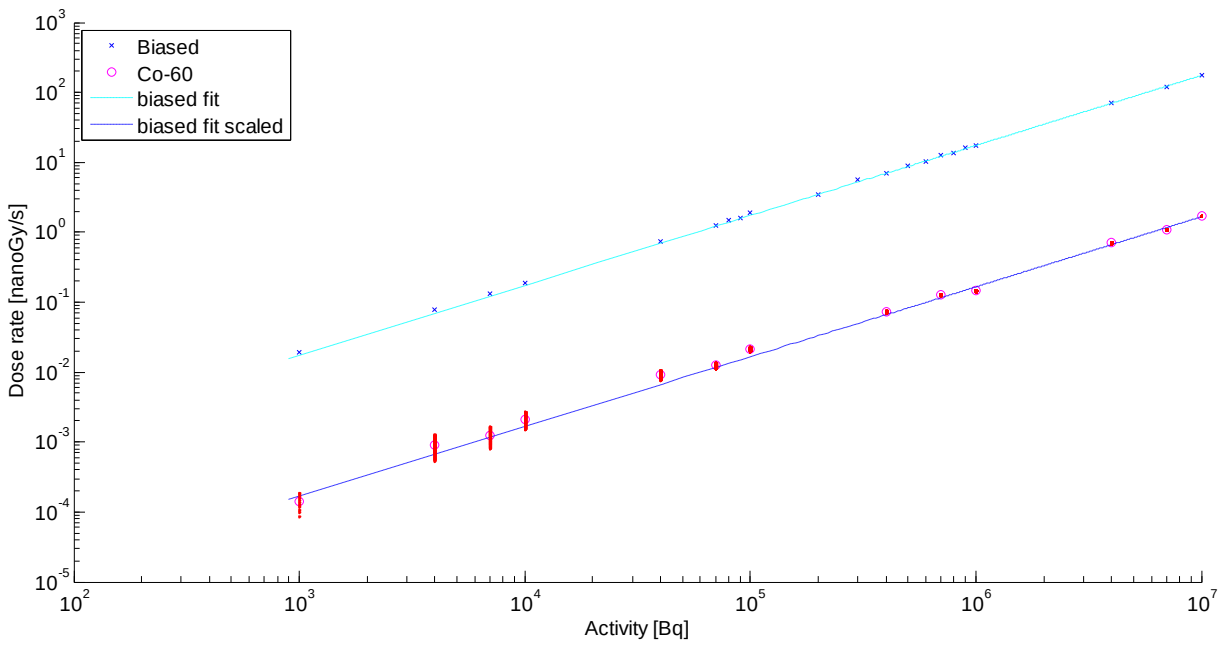

Figure 4. Dose rate (nGy/s) as a function of activity (Bq) for the biased and ^{60}Co simulations.

The fitted curves of the biased (scaled to compensate for the decreased solid angle) and ^{60}Co simulated data points are given by the following respective two equations, with dose rate in *nGy/s* and activity in *Bq*:

$$\dot{D} = \frac{0.1197}{4\pi}(174.2 \times 10^{-7}) \cdot A \tag{1}$$

$$\dot{D} = (1.663 \times 10^{-7}) \cdot A \tag{2}$$

The 95% confidence interval for the above dose rates can be obtained by multiplying $\dot{D}$ with 0.3%. Given in table 2 are the comparisons between the measured dose rate and those predicted by (1) and (2) when working with the documented activity (48.4 *TBq*) of the therapy unit's source; as well as the percentage difference between the measured and predicted value.

Table 2. Comparison of measured and simulated dose rate and difference between measured and simulated values.

	Dose rate (mGy/s)	% difference
Measured value	8.16	0
(1) Biased simulation	8.03 ± 0.03	1.6 ∓ 0.3
(2) ^{60}Co-simulation	8.05 ± 0.03	1.4 ∓ 0.3

4. Conclusion

Deviations in the CHO-K1 dose points exists, since only one slide per dose point was analyzed (due to time constraints) as well as human errors during the counting procedure (e.g. some count higher / lower than others). The α- and β-parameter values will be used in future studies regarding the relative biological effectiveness of Auger electrons from DNA-incorporated ^{123}I for these same cell lines.

The Monte Carlo simulations give the most accurate results when the decay of the source is simulated as comprehensively as possible; this however leads to an increase in computational time (213 *min* for a 10 *MBq* ^{60}Co-source). Overall, Geant4 produces results with an accuracy of at least 98%. This benchmarks the reliability of Geant4 for future work.

Acknowledgements

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