Cell irradiation dosimetry at the Portuguese Research Reactor in the frame of Boron Neutron Capture Therapy

Maxime Lamotte
maxime.lamotte@tecnico.ulisboa.pt
Instituto Superior Técnico, Universidade de Lisboa, Portugal
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Abstract

The present work aims to perform the dosimetry and microdosimetry of cell irradiations at the Portuguese Research Reactor (RPI), in the scope of ongoing research at IST-CTN for the development and *in-vitro* evaluation of novel Boron-loaded compounds for Boron Neutron Capture Therapy (BNCT). We report the dosimetric characterization of irradiation facility at the vertical access of the RPI thermal column. Experimental methods for dose measurement and monitoring of cell irradiations were based on neutron foil activation and thermoluminescent photon dosimetry. The Monte-Carlo particle transport simulation code MCNPX was used for a fine characterization of the mixed radiation field based on a detailed model of the irradiation facility coupled to an existing reactor core model. Simulations were extended to the evaluation of doses in cell structures using the Monte-Carlo GEANT4 code, aiming at a correlation with the observed cell damage.

At maximum reactor power, neutron fluence rates (averaged over the irradiation cavity) are ϕ_0=6.6x10^7 cm^{-2} s^{-1} (thermal) and θ=2.4x10^4 cm^{-2} s^{-1} with a photon dose rate of 150 mGyh^{-1}. These values agree with simulations within 85% (thermal neutrons) 78 % (epithermal neutrons) and 95% (photons) thereby validating the MCNPX model.

The GEANT4 simulations, based on a realistic cell model and measured boron concentrations, show that >95% of the dose in cells is due to the BNC reaction. A correlation with the radiobiology studies demonstrates that damage is mostly induced by the incorporated boron, with negligible contribution from the culture medium and adjacent cells, and evidence an extranuclear cell radiosensitivity.

1 Introduction

Glioblastoma multiforme (GBM) is a common type of malignant brain tumour. Despite progresses in robotic-surgery, radiotherapy and chemotherapy, the infiltrative shape and asymptomatic character at early development stage render glioblastoma a disease of poor prognostic with a median survival time after treatment of 10-12 months. BNCT has therefore emerged as an alternative treatment modality with a primary focus on GBM.

BNCT is a binary technique that relies on the combination of thermal (<0.5 eV) neutrons at the tumour site, and a boron-based compound with low cytotoxicity that has a preferential uptake by tumour relative to normal cells [1]. The compound becomes sensitised after neutron exposure due to the (n,α) nuclear reaction in the ^{10}B isotope (natural abundance 19.8%) which has a very large thermal cross-section (3870 barns) relative to other elements in tissue.

The BNC reaction is described as:

$$^{10}B + n \rightarrow {}^{4}He + {}^{7}Li + \gamma(0.48MeV) + 2.31MeV$$

(93.7%)

$$^{10}B + n \rightarrow {}^{4}He + {}^{7}Li + 2.79MeV$$ (6.3%)

The output energy is shared by the two fragments, with E(α)=1.87MeV and E(^{7}Li) =0.84MeV for the most probable reaction branch. These reaction

products are characterized by ranges in tissue comparable to cell dimensions (9 μm and 5 μm for α and Li, respectively), hence their energy is deposited within the boron-loaded cell, in close proximity of its nucleus, sparing the surrounding healthy tissues [2]. In addition, the Linear Energy Transfer (LET) (200keVμm^{-1} and 400keVμm^{-1} for α and Li, respectively) is in the range of maximum relative biological effectiveness (RBE) [2].

Although BNCT is generally considered as an experimental therapeutic modality at the stage of clinical trials, it has shown promising results for unresectable or incurable tumors such as GBM, head and neck malignancies and meningioma. Although the first trials started in the early 1960's, BNCT is not consensual in medicine. One of the main challenges with this therapeutic modality has been to find compounds with a high uptake in the tumor at a high tumor-to-normal selectivity allowing to deliver high doses to the tumor while keeping a reduced toxicity. It is generally considered that a good boron compound (for irradiations with a thermal neutron fluence of ~10^{12} cm^{-2} achievable in most reactors) should have a tumor uptake in the order of 20 ppm ^{10}B (corresponding to 10^9 ^{10}B atoms per cell) with a tumour-to-normal tissue concentration of 3 [3]. Up to now only two drugs are accepted for clinical use: boronophenylalanine (BPA) and sodium borocaptate (BSH). However, their low selectivity, reduced penetration in the cell nuclei, inability to cross the blood-to-brain cancer and reduced solubility in water yields them relatively inefficient [4].

The RPI has been involved in several activities in the context of BNCT since the 1990's with several collaborations with national and European institutions. The configuration of the irradiation facility at the vertical access of the thermal column was optimized with a Lead shield in order to reduce the gamma dose, and this facility has been used for thermal neutron irradiation of cell cultures (mainly melanoma A2058 cells loaded with BPA) and small animals [5], [6]. An epithermal beam facility was designed for further BNCT experiments involving animals, including *in-vivo* measurements of boron concentrations [7], [8]. After a time gap of 10 years experiments are now progressing with other cell types representative of cancer diseases with poor prognosis and particularly with novel compounds [9], [10].

The new compounds studied in this paper belong to the so-called 3rd generation boron delivery agents, and rely on boron atomic clusters in order to carry more atoms (up to 10 per molecule) in a non-cytotoxic compound. Dodecarborane appears as a good cluster as it remains stable in biological environment. A DNA-binding agent, associated with tumour-targeting molecules and boron clusters should strongly improve the efficiency of BNCT over the classical boron agents [11].

2 Materials and methods

2.1 The irradiation facility

The RPI is a 1 MW (thermal power) pool-type reactor, built by AMF Atomics (USA) and commissioned in 1961. Core conversion to low-enriched fuel (^{235}U~20%) was concluded in 2007 with the support of a Monte Carlo model of the reactor core, validated later for the determination of neutron fluence rates.

Several facilities are available for irradiations with fast, epithermal and thermal neutrons. The thermal column consists of a graphite stacking that moderates neutrons from the core with minimal absorption. Cell irradiations were performed in the vertical access of the thermal column, which resembles a well with a cavity at the bottom that is very convenient for the irradiation of monolayer cell cultures.

The irradiation cavity is displaced vertically from the fuel upper level, at a distance of ~3m from

the core with an overall graphite thickness of ~1.5 m. The thermal neutron fluence rate at 1 MW is in the order of $7x10^7$ $cm^{-2}s^{-1}$. It is approximately constant in the direction parallel to the core, whereas a fluence gradient exists in the perpendicular direction, i.e., at different distances from the core.

2.2 Cell irradiations

Three boron compounds (carboranylmethylbenzoacridones) based on boron clusters and DNA intercalators were investigated in this work [12]. Previous to irradiation, the glioblastoma U87 cells were incubated for 30 minutes in cell medium (~ 10^5 cells/200 µL) with the compound at concentrations ranging from 50 to 200µM.

The cells were placed in a flat-surfaced polystyrene cell culture flask of 25cm^2. Each flask contained a monolayer of U87, filled with 5 mL medium up to 2 mm high. The flasks were laid on a Plexiglas box (hereafter, irradiation box) allowing the simultaneous irradiation of 2 rows of 6 flasks, each row at constant distance from the core yielding similar exposure conditions for its flasks.

Five cell irradiations were performed, at 600 kW with a thermal neutron fluence rate of 3.8 to 5.1 $x10^7$ $n_{th}cm^{-2}s^{-1}$. Irradiation times ranged from 2h to 5h in order to achieve different cell doses. Each evaluation comprised a minimum of two flasks irradiated in the same row - one with the compound and a control without the compound – and a non-irradiated blank.

2.3 Dose measurements

2.3.1 Activation foils

Pure gold foils (Ø 6mm x 50µm) were employed to measure and map thermal and epithermal neutron fluence rates in the irradiation box, and also to monitor the neutron fluence in each cell irradiation. The activity induced by the radiative capture reaction $^{197}Au(n,\gamma)^{198}Au$ (half-life: 2.7d, thermal neutron cross section: 98b, resonance integral: 1562b) was measured by gamma spectroscopy using a HPGe detector calibrated for energy and efficiency.

The Cadmium ratio method was used to discriminate the activation induced by thermal and epithermal neutrons. This method is based on the fact that a Cd filter of 1 mm thickness acts as a high-pass filter at 0.5 eV; the irradiation of bare and Cd-covered foils allows to determine the epithermal (from the activation of the Cd-covered foil) and thermal (by the difference between the responses of bare and covered foils) fluence rates.

Irradiation monitoring was achieved with 4 foils, each placed at a fixed position in the corner of the irradiation box, in order to reduce the perturbation induced by the cell flasks or Cd covers.

2.3.2 Thermoluminescence dosimetry

The photon dose was measured by thermoluminescence dosimetry (TLD) using two different materials of low thermal neutron sensitivity: Al_2O_3:C and ^{7}LiF:CuPMg TLDs (Thermo Scientific TLD-500 and TLD-700H, respectively) with an estimated relative thermal neutron sensitivity of 3.5 mGy/10^{10} cm^{-2} [13]. These were used in pairs at each measurement point. As TLD-500 is sensitive to light, all dosemeters were protected from light during storage and irradiation.

The TLDs were calibrated individually for kerma in air using a ^{60}Co source at the metrology laboratory of IST/CTN. The calibration was performed with 100mGy, with a field size of (14.4 x 14.4)cm at 1 meter from the source corresponding to a kerma rate of 750 µGys^{-1}. A set of 31 TLDs was irradiated simultaneously in an opaque Plexiglas support of 5 mm thickness.

The TLDs were measured 1 day after irradiation in order to reduce fading effects. Control

dosimeters exposed in a $^{90}Sr/Y$ irradiator were used to monitor possible sensitivity fluctuations.

2.4 MCNPX characterization of the irradiation facility

A detailed study of the radiation fields at the vertical access of the thermal column was performed in order to address the contamination of the thermal neutron field by gamma rays, epithermal and fast neutrons. A computer-aided design (CAD) model of the facility's geometry was built and exported to the input file of MCNPX. The graphite density was measured on an original spare block and a Boron-equivalent contamination up to 10ppm was allowed [14].

A surface neutron source at 25 cm from the core was extracted from a criticality run of the core model and coupled to the simulation.

The large distance and vertical displacement from the core with a very thick moderator render Monte Carlo simulations difficult due to the reduced fraction of source neutrons that reach the irradiation cavity. Variance reduction techniques were necessary to achieve a good statistical uncertainty and reduce calculation time. Mesh weight windows were employed considering a superimposed mesh with dimensions comparable to the neutron track length in each material. The MCNPX weight generator was used to obtain indicative weights, that were adjusted manually in order to attain a progressive weight increase from the core to the cavity.

Neutron and photon spectra and doses were calculated at the surface of the irradiation box. Dose calculations were based on flux-to-dose-rate conversion factors: kerma factors for neutrons and mass absorption coefficients for photons.

2.5 GEANT4 simulations of cell doses

Cell doses were calculated with GEANT4 due its high customization potential, the ability to track primary and secondary particles and furthermore with an eye on the GEANT4-DNA package for future DNA damage simulation [15] [16].

In addition to the dose deposited by the BNC reaction (the alpha dose), the following dose components are normally considered: (i) thermal (<0.5 eV) neutron dose, from the production of 580 keV protons following the $^{14}N(n_{th},p)^{14}C$ reaction; (ii) fast (0.1-10 MeV) neutron dose, through the proton recoils following neutron scattering in hydrogen $^{1}H(n,n)^{1}H$; (iii) photon dose, from radiative capture reactions namely in H. Epithermal neutrons (0.5 eV-100 keV) usually add little to the non-therapeutic dose.

For average cell dose calculations, the geometry model considered a flask in the irradiation box and its Lead shield. A cell-containing layer of 200μm height was included at the bottom of the flask, with a homogeneous mass composition of 20% water, 80% brain cells (their elemental composition given by ICRU report 44 [17]) and 25 ppm of natural B. The layer volume and mass are 0.5 mL and 0.5132 g, respectively.

Neutrons in three energy groups (thermal, epithermal and fast), as well as photons, were considered separately in the source term, their angular and energy distribution retrieved by the MCNPX simulations. The simulation outputs the energy deposited in the cell layer by each kind of primary particles (neutrons and photons) and secondary particles (alphas, protons, electrons, photons and other nuclear reaction products). This discrimination allowed to identify the interactions and deposited energy induced by each group.

Upon finding that most cell dose was induced by the BNC reaction, dose calculations in the cell structures considered only thermal neutrons field. Bi- and tri-dimensional cell clusters were simulated with a simplified spherical cell model consisting of a nucleus embedded in cytosol+cytoplasm+membrane

material, at Boron concentration levels determined by inductively-coupled plasma mass spectrometry (ICP-MS).

2.6 Radiotoxicity

After irradiation cytotoxicity assays and biomarkers of cell death assays were performed. The compounds are fluorescent which permits to visualize its distribution inside the cells. The assays were subdivided in two categories of early and late damages.

A (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTT) assay measured the metabolic activity of the living cells which can be correlated with the number of cells. The population death rate was assessed by MTT after the irradiations to quantify the early damages of the BNC experiment and their impact on the cell population.

Double Strand Breaks (DSB), is a good indicator for early damages induced by particles or chemicals in the DNA, they were estimated by the H2AX gene assay. An accumulation of H2AX gene is noticeable around DSB, and a fluorescent stain allows to visually notice such build-up, and so of DSB.

To correlate the DSB occurrence with the boron compound uptake, a fluorescent stain was added in the compound, allowing to determine the spatial distribution of the carboranylmethylbenzoacridone inside the cells under ultraviolet light.

Late damages were characterized by the mean of micronucleus assay. During the mitosis of a cell, more than two nuclei (usually much smaller) can appear, translating a chromosomic damage. The occurrence of micronucleus was counted on control and irradiated cells to estimate the late damages engendered by the boron capture reactions.

3 Results and Discussion

3.1 Characterisation of the irradiation facility

The surface neutron source employed in the simulations contained 40.7 million neutron histories. The simulation of the neutron transport through the thermal column was performed in a 32-core, 64GB RAM supercomputer, with a typical run times of 2.6 day. A Boron-equivalent contamination of 10 ppm was set for the simulations, as it yielded the best agreement between calculated and measured thermal neutron fluences with negligible influence over epithermal fluence rate. This contamination may be representative of the actual B in graphite associated to other elements normally found in graphite and in Lead shields.

The total neutron fluence at the irradiation facility is composed of 0.11% (fast), 0.21% (epithermal) and 99.67% (thermal neutrons). The discrepancy between calculated and measured fluence and dose rates, averaged over the surface of the irradiation box (Table 1), was +15% (thermal neutrons), -22% (epithermal neutrons) and -5% (photons).

A systematically higher photon dose (by 14%) has been measured with TLD-700H over TLD-500 over 15 points, which can be due to the application of nominal neutron sensitivity values. From this, an uncertainty of 10% is estimated for the photon dose measurements. The homogeneity of the neutron fluence and photon dose in the transversal

Table 1 Simulated and measured thermal neutron fluence rate (ϕ_0), epithermal neutron fluence rate per unit lethargy (θ), and gamma dose rate (D'_γ) with respective relative uncertainties (ε).

	$\phi_0 (ncm^{-2}s^{-1})$	ε (%)	$\theta (ncm^{-2}s^{-1})$	ε (%)	$D'_\gamma (mGyh^{-1})$	ε (%)
simulated	7.59E+7	4.31	1.96E+4	9.64	151.0	1.50
measured	6.55E+7	3.00	2.40E+4	3.00	158.6	7.56

direction is better than 3% and 6%, respectively. The consistency of the irradiation monitoring results is 97%, as determined upon 11 experiments. The general shape of the measured longitudinal neutron and photon profiles was reproduced by the simulations.

3.2 Cell dose components

About 10^5 neutrons in each energy group (thermal, epithermal and fast) were generated, in addition to 10^6 photons, leading to a total of 462 interactions within the cell culture layer.

Table 2 shows the contribution of the various radiations to the cell dose. The energy deposited by the incident photon component is negligible, similarly to the epithermal and fast neutron contributions. The BNC reaction induces >95% of the total dose. The dose rate at 1 MW reactor power is 0.602mGyh^{-1}. If higher (>25 ppm) boron concentrations are encountered, the contribution of the BNC to the dose will be even higher, as fast and epithermal neutrons, as well of photon reactions are independent to the boron amount in the culture.

3.3 Doses in cell structures

The simulation considered 10^6 thermal neutrons incident on a layer of fifteen U87 cells (within a volume of 100μm^3). The doses received by the nucleus and cytoplasm of a single cell were calculated, in conditions similar to the actual experiment. The dose received by the central cell is about 60Gy for a thermal neutron flux of $4x10^{12}$ n.cm^{-2}, and a total cell boron uptake of 1187ppm. Alpha and Lithium fragments produced in adjacent cells do not induce an appreciable dose in the cells. In an extreme situation of a cell medium containing a high amount of boron (1% in mass), the dose in the cytoplasm is increased by only 0.8%. The dose in the cell is therefore induced mainly from the incorporated compound.

3.4 Radiotoxicity

MTT assays performed 72h after irradiation showed a cell death 80% higher (relative to the blank) in cultures incubated with 200μM carboranylmethylbenzoacridone and exposed to a thermal neutron fluence of $8.3x10^{11}$ $n_{th}cm^{-2}$.

Early damages are particularly visible in cells containing the compound in the cytosol, cytoskeleton or nucleus, where several double strand breaks are revealed by the γH2AX assay, immediately after irradiation.

Late damages, quantified by the micronucleus assay displayed a clear impact of the irradiation on the cell division capability, with a micronucleus occurrence of 25% of the surviving cell population vs. 4% on unirradiated cell cultures.

A comparison of results obtained with the various compounds showed that a predominant incorporation of the compound in the nucleus is not critical for the effectiveness of the BNC reaction, in contrast to early assumptions and corroborated by independent studies [18].

Table 2 Contribution of the components of the mixed field to the overall dose in the cell layer (Gy/ncm^{-2})

	Cell average		*Cell nucleus*		*Cell cytosplasm&cytosol*	
Boron distr.	D(Gy)	ε(%)	D(Gy)	ε(%)	D(Gy)	ε(%)
One cell	59	5.98	36.8	11.95	58.7	5.72
All cells	60.5	5.75	35.7	12.22	56.4	5.78

Table 3 GEANT4 simulation dose results of the microdosimetric mode in different configurations(D) with respective relative uncertainty (ε)

	^{7}Li	*electrons*	*alphas*	*gammas*	*protons*	*nuclei*	*TOTAL*	*Contribution*
n_{th}	7.70E-13	3.59E-15	1.34E-12	3.37E-16	8.63E-21	5.59E-22	2.11E-12	95.87
n_{epi}	7.95E-15	8.81E-18	1.39E-14	2.51E-18	4.38E-21	5.14E-23	2.19E-14	0.99
n_{fast}	1.71E-14	1.27E-15	2.01E-14	0.01%	1.71E-14	1.27E-15	2.01E-14	0.91
γ	0.00E+00	4.90E-14	0.00E+00	5.88E-19	0.00E+00	0.00E+00	4.90E-14	2.22

4 Conclusion

A Monte Carlo model of the thermal column of the RPI has been developed and employed towards the investigation of the radiation field at the facility. A 10ppm Boron-equivalent contamination of the

graphite was determined upon comparison with activation measurement. Simulated and measured outputs within ±15%, ±22% and ±5% for thermal neutrons, epithermal neutron and photons, respectively. This level of agreement is considered satisfactory in integral neutron fluence and photon dose measurements for reactor dosimetry. The Monte Carlo model of the experimental setup shows that the BNC reaction contributes with more than 95% of cell doses, allowing to simplify the irradiation dosimetry and microdosimetric model.

From microdosimetric simulations, the dose received by each cell with a compound concentration of 350μM and exposed to a thermal neutron fluence of 8.20×10^{11} $n_{th}cm^{-2}$ (corresponding to an irradiation at maximum reactor power for ~3h) is 12Gy.

The carboranylmethylbenzoacridone compounds employed in this work have low cytotoxicity. Some are able to carry large amounts of boron (up to 2.06×10^{10} atoms of ^{10}B) into the cell. For these, a population death reaching 80% over non-irradiated and/or non-incubated cell cultures. The incorporation of the compound into the cell is a necessary condition to achieve radiotoxicity. Once this requirement is satisfied the effectiveness of the BNC reaction is undeniable. The assimilation of the compound by the nucleus does not seem to be critical, as double strand breaks and late damage are observed in its absence while still incorporated in other cell compartments.

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