Experimental report

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Proposal: 3-07-388 **Council:** 4/2019

Title: Neutron radiobiology data for BNCTby cell irradiation

Research area: Other...

This proposal is a continuation of 3-07-376

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Samples: mammalian cells

Instrument	Requested days	Allocated days	From	To
PF1B	10	10	05/06/2021	18/06/2021

Abstract:

This experiment has the purpose to continue with the series of experiments performed at the Pf1b to obtain biological effect of different cell lines after neutron irradiation and boron compound testing. After good results in 5 cell lines and nitrogen labeling, the intention is to work with more cell lines and to focus more on the boron compound trials.

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INSTITUTE LAUE-LANGEVIN EXPERIMENTAL REPORT

Experiment 3-07-388: Neutron radiobiology data for BNCT by cell irradiation

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Objective

Neutron radiobiology data are critical for an accurate treatment planning in Boron Neutron Capture Therapy. The determination of the relative biological effectiveness (RBE) for neutrons with respect to photons is very important for neutron capture cancer treatments. They are required for predicting the expected outcome of the treatment (equivalent photon dose delivered to the tumor) as well as for determining the treatment planning (maximum equivalent photon dose delivered to healthy organs). Also, in conventional radiotherapy with LINACS, the estimation of the dose delivered to peripheral organs due to the secondary neutrons, which is a problem of recent concern, requires these values.

The aim of this experiment was to complete the dose-response data obtained in previous measurements at ILL (3-07-376) in order to achieve sufficient statistics for obtaining more precise RBE data. For that purpose, we irradiated 5 different cell lines (tumor and normal cells) at the cold neutron beam at PF1b, where the gamma contamination is negligible, and only photons produced in the culture media by radiative neutron capture by hydrogen affects the cells, and can be subtracted as they are not the main dose component.

In addition, more tests of the effect of the nitrogen capture, the main reaction occurred at low energies, separated from the hydrogen capture effect, were done by isotope labeling. This has been a challenging task, and was done by growing the cells in a special medium where ^{14}N has been replaced by ^{15}N.

Experimental setup

The irradiation of the culture cells were performed at the very pure cold neutron line PF1b, thanks to its bent guide which prevents contamination from fast neutrons or photons from the reactor core.

The cells were grown in quartz cuvettes, very thin to avoid as much as possible secondary photons from the culture medium. This medium has just a thickness of 2 mm (inner space of the cuvettes). Quarts is very transparent to neutrons, which avoids activation and secondary radiation.

The cuvettes were place at the beam exit as illustrated in Fig. 1.

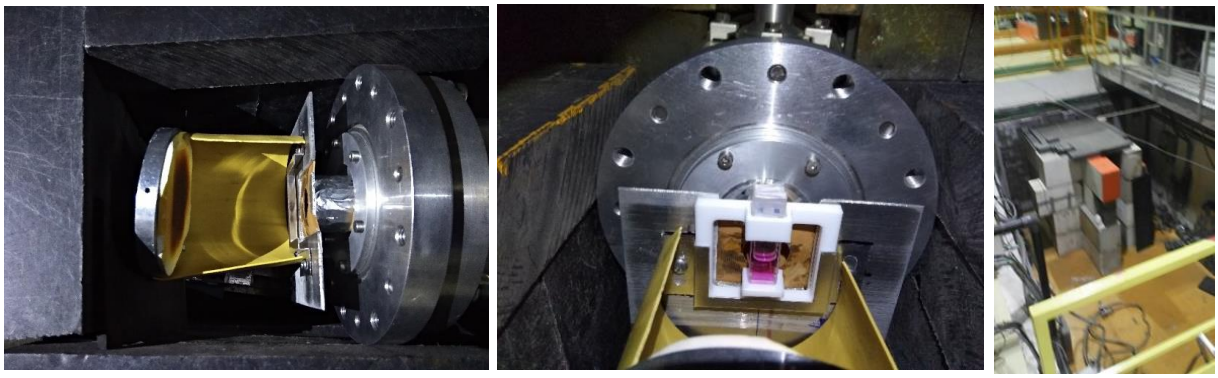

Fig. 1. *Set up of the experiments. Left: top view of the beam exit (on the right in the picture), the beam stop (on the left). Center: view of the set-up with the sample holder and the quartz cuvettes containing the cell culture (culture medium is pink). Right: casemate shielding of concrete and lead.*

Sample holder and shielding

The sample holder is made of Teflon (to avoid activation) and has the space to insert the quartz cuvettes (two at the same time). A Li6 sheet is situated 2 cm after the holder, to use as a beam stop. Then, a lithium sheet is placed around the sample holder, surrendered also with lead bricks with a boron layer. A concrete structure shields the whole experimental area.

Cells

The tumor cell lines used were: A375 (Malignant melanoma), Cal33 (tongue squamous cell carcinoma) and A172 (glioblastoma). The normal tissue cell lines used are: Hek293 (embryotic kidney) and CCD10 (skin). Cells were incubated 24 hours before irradiation inside quartz cuvette. For the irradiation, the cuvettes are placed in the teflon holder. After irradiation the cells are recovered from the cuvettes with trypsin, counted and prepared for survival assays.

Measurements

The neutron flux was measured from with a Gold foil and a Ge detector. The dose rate for the beam at the cells (both neutron and photon) was estimated from Monte Carlo simulations with MCNP v.6.2 with the geometry of the cell-containing cuvettes boxes.

The preparation of the samples was done daily at the P2 LAB at ILL. Then the whole set was moved to PFB1 for the irradiation. Each sample was irradiated sequencially at different times of the set, and one was left without irradiation as control. After waiting some minutes because of minor activation, they were moved in the evening to the Bio Lab. 4 hours after irradiation, inmediate death was measured by counting the fraction of live cells with a cellometer, some cells of each samples were prepared for proliferation analysis with resazurin and for clonogenic assays in the following days.

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Results

	A172					
	Survival (mean)	err S	irrad time (min)	Dose (Gy)	ln S	error LnS
C	1	0,289506624	0	0	0	0,289507
Q1 70min	0,060860311	0,018716989	70,05833333	3,687839872	-2,799174027	0,30754
Q2 70min	0,109982922	0,045311948	70,05833333	2,087122436	-2,207430175	0,411991
Q1 33min	0,16767781	0,059367056	32,825	1,727893571	-1,785710938	0,354054
Q2 33min	0,393268497	0,111033967	32,825	0,977896429	-0,933262703	0,282336

	A172+BPA					
	Survival (mean)	err S	irrad time (min)	Dose (Gy)	ln S	error LnS
C	1	0,27686028	0	0	0	0,27686028
Q1 7min	0,008576473	0,00306905	7,033333333	3,531936972	-4,758732528	0,35784495
Q2 7min	0,009751773	0,00323921	7,033333333	1,689147328	-4,630306159	0,33216665
Q1 3min	0,013544983	0,00527435	3,05	1,531621957	-4,301739039	0,38939514
Q2 3min	0,040529767	0,02221093	3,05	0,732497538	-3,205718594	0,54801531
Q1 1min	0,142412275	0,06712389	1,011111111	0,507750813	-1,949029085	0,47133497
Q2 1min	0,411573964	0,13283671	1,011111111	0,242831606	-0,887766533	0,32275294

	Cal33+BPA					
	Survival (mean)	err S	irrad time (min)	Dose (Gy)	ln S	error LnS
C	1	0,29484387	0	0	0	0,29484387
Q1 7min	0,010549242	0,00390876	7,1	3,453019331	-4,55170129	0,3705249
Q2 7min	0,010106237	0,00521095	7,1	1,652559223	-4,59460251	0,51561752
Q1 3min	0,014848682	0,00586633	3,016666667	1,467127932	-4,20984415	0,39507413
Q2 3min	0,030254427	0,01699658	3,016666667	0,702143707	-3,49811275	0,56178803
Q1 1min	0,089680337	0,03809903	1,038888889	0,505254002	-2,41150374	0,42483147
Q2 1min	0,273042436	0,1237438	1,038888889	0,241806396	-1,29812805	0,45320354

	A375 + BPA			
	Survival (mean)	err S	irrad time (min)	Dose (Gy)
C	1	0	0	0
Q1 7min	0,011786372	7	3,823670576	-4,4408113
Q2 7min	0,028176796	7	1,825501009	-3,5692565
Q1 3min	0,077348066	2,983333333	1,629611984	-2,5594397
Q2 3min	0,213627993	2,983333333	0,778011144	-1,5435191
Q1 1min	0,556169429	0,983333333	0,537134676	-0,5866823
Q2 1min	0,82320442	0,983333333	0,256439427	-0,1945507

	CCD10					
	Survival (mean)	err S	irrad time (min)	Dose (Gy)	ln S	error LnS
C	1	0,1674104	0	0	0	0,1674104
Q1 75min	0,111791484	0,02739034	74,98888889	3,565977123	-2,1911199	0,24501277
Q2 75min	0,226277264	0,03436727	74,98888889	2,055519609	-1,4859942	0,15188122
Q1 60min	0,150342536	0,03600564	59,71111111	2,839466745	-1,894839	0,23949072
Q2 60min	0,435640807	0,05247252	59,71111111	1,636740611	-0,8309372	0,12044904
Q1 15min	0,650113122	0,1587738	14,99444444	0,713036913	-0,4306089	0,24422489
Q2 15min	0,759991639	0,21001677	14,99444444	0,411012552	-0,2744478	0,2763409

	CCD10 + BPA					
	Survival (mean)	err S	irrad time (min)	Dose (Gy)	ln S	error LnS
C	1	0,355825442	0	0	0	0,35582544
Q1 10min	0,030395517	0,013436333	9,916666667	3,41848659	-3,4934601	0,4420498
Q2 10min	0,03305564	0,013966647	9,916666667	1,650923938	-3,4095631	0,42251935
Q1 6min	0,03253688	0,015779751	6,011111111	2,072158258	-3,4253811	0,48498047
Q2 6min	0,028769175	0,016928451	6,011111111	1,000728124	-3,5484508	0,58842323
Q1 3min	0,027609007	0,017632991	3,016666667	1,039909366	-3,5896132	0,63866808
Q2 3min	0,059200444	0,034930802	3,016666667	0,502213837	-2,8268262	0,59004291

	Hek293 + BPA					
	Survival (mean)	err S	irrad time (min)	Dose (Gy)	ln S	error LnS
C	1	0,48303472	0	0	0	0,48303472
Q1 7min	0,01063631	0,00728643	7	2,499116276	-4,5434817	0,68505221
Q2 7min	0,011621361	0,00849239	7	1,205635696	-4,4549104	0,73075666
Q1 3min	0,00943777	0,00914297	3	1,071049833	-4,6630355	0,96876378
Q2 3min	0,046912313	0,02944292	3	0,516701013	-3,0594751	0,627616
Q1 1min	0,083099119	0,03582997	0,988888889	0,35304976	-2,4877212	0,43117147
Q2 1min	0,454448755	0,20480938	0,988888889	0,170319963	-0,7886701	0,45067652

	CHO					
	Survival (mean)	err S	irrad time (min)	Dose (Gy)	ln S	error LnS
C	1	0,2536941	0	0	0	0,2536941
Q1 75min	0,067333677	0,01686713	74,92222222	4,538870554	-2,6980948	0,25050069
Q2 75min	0,28086937	0,05280136	74,92222222	2,510470769	-1,2698656	0,18799258
Q1 60min	0,128102827	0,03723203	60,00555556	3,635202496	-2,054922	0,29064177
Q2 60min	0,390581563	0,10119553	60,00555556	2,010647692	-0,9401185	0,25908937
Q1 15min	0,824303501	0,17992109	15,00555556	0,909053045	-0,1932165	0,21827044
Q2 15min	0,875115924	0,22632796	15,00555556	0,502801538	-0,1333989	0,25862626

		CHO + BPA				
		Survival (mean)	err S	irrad time (min)	Dose (Gy)	error LnS
C	1	0,37721213	0	0	0	0,37721213
Q1 7min	0,016898601	0,01048645	7,005555556	3,720440711	-4,0805244	0,62055156
Q2 7min	0,038190911	0,02043662	7,005555556	1,777220115	-3,2651577	0,53511746
Q1 3min	0,051461187	0,02475232	2,994444444	1,590259749	-2,9669274	0,48099
Q2 3min	0,103895774	0,04421585	2,994444444	0,759652373	-2,2643671	0,4255789
Q1 1min	0,167355222	0,09726501	1	0,531070046	-1,7876366	0,58118896
Q2 1min	0,450815395	0,14431476	1	0,253687249	-0,7966973	0,32011942