Life Sciences in Space Research 16 (2018) 38–46

Contents lists available at ScienceDirect

Life Sciences in Space Research

journal homepage: www.elsevier.com/locate/lssr

An innovative in vitro device providing continuous low doses of γ-rays mimicking exposure to the space environment: A dosimetric study

V. Pereda-Loth[a,*], X. Franceries[b], A.S. Afonso[b,c], A. Ayala[b,c], B. Eche[c], D. Ginibrière[a], G. Gauquelin-Koch[d], M. Bardiès[b], L. Lacoste-Collin[c], M. Courtade-Saïdi[a,c]

[a] Université de Toulouse III-Paul Sabatier, GSBMS, UMR5288, MedEvo, Faculté de Médecine, 37 Allées Jules Guesde, F-31073 Toulouse Cedex 3, France
[b] Université Toulouse III-Paul Sabatier, Inserm, UMR1037 CRCT, Toulouse, F-31000, France
[c] Laboratoire d'Histologie- Embryologie, Faculté de Médecine de Rangueil, 133, Route de Narbonne, F-31062 Toulouse Cedex 9, France
[d] Centre National d'Etudes Spatiales, 2 Place Maurice Quentin, 75039 Paris Cedex 01, France

ARTICLE INFO

Keywords:
Low-dose radiation
Simulated microgravity
Random positioning machine
Space biology

ABSTRACT

Astronauts are exposed to microgravity and chronic irradiation but experimental conditions combining these two factors are difficult to reproduce on earth. We have created an experimental device able to combine chronic irradiation and altered gravity that may be used for cell cultures or plant models in a ground based facility.

Irradiation was provided by thorium nitrate powder, conditioned so as to constitute a sealed source that could be placed in an incubator. Cell plates or plant seedlings could be placed in direct contact with the source or at various distances above it. Moreover, a random positioning machine (RPM) could be positioned on the source to simulate microgravity. The activity of the source was established using the Bateman formula. The spectrum of the source, calculated according to the natural decrease of radioactivity and the gamma spectrometry, showed very good adequacy. The experimental fluence was close to the theoretical fluence evaluation, attesting its uniform distribution. A Monte Carlo model of the irradiation device was processed by GATE code. Dosimetry was performed with radiophotoluminescent dosimeters exposed for one month at different locations (x and y axes) in various cell culture conditions. Using the RPM placed on the source, we reached a mean absorbed dose of gamma rays of (0.33 ± 0.17) mSv per day.

In conclusion, we have elaborated an innovative device allowing chronic radiation exposure to be combined with altered gravity. Given the limited access to the International Space Station, this device could be useful to researchers interested in the field of space biology.

1. Introduction

Space experiments involving biological investigations have generated considerable interest worldwide. Many of them have been scheduled for the established experimental area in the International Space Station (ISS), where astronauts and experimental subjects are exposed both to space radiation and to microgravity. These combined conditions are difficult to simulate at ground level. NASA and ESA have highlighted the need to focus on the biological effects of protons and heavy ions, together with secondary radiation, on astronauts who are in space mission (ISS, Moon, Mars) (NASA, 2009; ESA, 2003). However, primary cosmic heavy particles do not necessarily constitute a major component of spatial radiation. As mentioned by Maalouf et al, in an experiment using cells thawed and cultured after 133 days of ISS mission (Ohnishi et al., 2009), they only observed about 1.5 tracks of γ-H2AX foci per 100 cells. Although the authors suggested that the majority of tracks might have been caused by high-LET ions, the relative contribution to the total dose of particles with LET more than 10 keV per μm was only 6.2% (Maalouf, et al., 2011; Ohnishi et al., 2009). On the contrary, an effort should be made to continue unravelling the basic mechanisms specific to low-dose, to repeated doses and to adaptive response, which no space shield can protect from.

It is now well demonstrated that when cells, organs, or entire organisms are exposed to radiation at low doses or at low dose rates, an adaptive response may be observed. Such adaptive response include a decrease of radiation-induced damages such as cell death, gene mutations, micronuclei formation, chromosome aberrations, and malignant transformation, especially after a challenging higher dose (Elmore et al., 2008; Ghiassi-nejad et al., 2002; Kojima et al., 2000; Mitchel et al., 1999; Sanderson and Morley, 1986). For example, it has been reported that a priming low-dose exposure, or an exposure with low-dose rate, can induce a p53 tumour suppressor gene-dependent

* Corresponding author.
E-mail address: veronica.pereda@univ-tlse3.fr (V. Pereda-Loth).

http://dx.doi.org/10.1016/j.lssr.2017.10.004
Received 27 January 2017; Received in revised form 25 September 2017; Accepted 24 October 2017

adaptive response in mammalian cells (Takahashi et al., 2010).

This observation is of interest because the radiation environment in space includes low doses and low dose rates. The daily dose received in the ISS has been estimated at 0.5 mSv, assessed by physical dosimetry using phantoms (Cucinotta et al., 2008).

The magnitude of the Earth's gravity vector cannot be changed but its effect on biological systems can be modified and microgravity simulators may generate functional near weightlessness of a cell or organism (Briegleb, 1992). These kind of devices are very useful for studying the effect of altered gravity on biological organisms or to prepare and/or complete a study that could be selected to be performed on the ISS. One of these devices is the “random positioning machine” (RPM), which is characterized by the randomly changing rotational speed and 3D-direction of a platform where biological samples can be placed (Borst and van Loon, 2009). A recent review suggests the use of 3D rotation to better simulate microgravity in *Arabidopsis*. However, fast 2D rotation seems to be more suitable for cells or other organisms requiring a large liquid volume (e.g. *Euglena, Chara*) in order to avoid shear forces within the volume (Herranz et al., 2013).

Here, we present an original device combining both simulated microgravity and continuous low dose radiation using an RPM, which may be adapted also to clinostat to better simulate microgravity depending on the selected model organism for a specific experiment. The most innovative part of our device is that it delivers very low-dose rates, allowing *continuous* irradiation during the whole experiment (several days) simulating better the space environment (300 μSv per day at the ISS (Vanhavere et al., 2008)).

Only few interesting devices combining microgravity and low-dose radiation already are available worldwide. For example, Ikeda et al. use an RPM combined with an X-generator that generates pulse-irradiation are also an option for simulating space environment. However, tested X-irradiation protocol delivers average doses of 0.036 Gy/min by irradiating samples 29 times every minute during 30 min (Ikeda et al., 2016). Therefore, in order to simulate space environment as in the ISS for example, this device would only provide very few pulse-radiation (*discontinuous model)* to rapidly reach the maximum dose rate/day.

Another interesting device developed by Ikea et al. (2017), using a three-dimensional clinostat, is synchronized to a heavy-ion irradiation system that is very suitable for studies focusing on the effects of high-energy charged particles (Ikea et al, 2017).

2. Material and methods

2.1. Presentation of the combined irradiation and gravity alteration device

In vitro continuous irradiation was provided by thorium nitrate, a crystalline salt having the formula $Th(NO_3)_4 \cdot xH_2O$. Thorium has 13 isotopes (atomic masses ranging from 212 to 236, Fig. 2), of which the main one is ^{232}Th, an emitter of alpha radiation with 4 MeV energy and gamma radiation with 60 keV energy (Table 1), according to the IAEA (International Atomic Energy Agency, 2016). Thorium is a natural radioactive element, contributing to the telluric external exposure. It belongs to the actinide series and is present in radioactive disintegration chains of the natural (4^{n+0} family). Its half-life is 1.405×10^{10} years and it thus ensured a constant rate of irradiation throughout the experiment (Argonne National Laboratory, 2016).

The thorium nitrate powder was contained in three layers of airtight plastic bags 33×36.5 cm^2 with a total thickness of 0.4 to 1.2 cm. The whole was enclosed in a protective cardboard envelope 32×44 cm^2, itself covered with an airtight plastic bag. This device could be treated as a sealed source and was placed in a cell incubator. Petri dishes were positioned in contact with the source for biological experiments, which have been described previously (Lacoste-Collin et al., 2015, 2011).

The random positioning machine, a device simulating microgravity, can be programmed to simulate truly random directions, speeds and intervals of speed changes. Culture flasks, petri dishes o culture chambers can be placed on the RPM platforms. Our RPM is a desktop instrument developed by the Dutch Space (P.O Box 32070 2303 DB Leiden The Netherlands), model #2001-. This desktop RPM contains two perpendicular cardanic frames, with an experiment platform, that are moved according to a 'random walk scenario' generated by software in a PC (Huijser, 2000). A video of this desktop RPM is available for better understanding of this instrument (Supplemental data).

This device, combining thorium nitrate irradiation and a microgravity simulator, is presented in Fig. 1.

Table 1
Information on emission decay from the IAEA (International Atomic Energy Agency) and MIRD (committee on Medical Internal Radiation Dose) (Eckerman et al., 2008).

Half-life (years = a)	Nuclide	Decay mode					
		α Energy released		β-		γ	
		keV	%	keV	%	keV	%
		4012.3	78.2			63.81	0.263
1.405E+10 a	Th-232	3947.2	21.7			140.88	0.021
		3811.1	0.069				
5.753 a	Ra-228			39.2	40	15.15	
				12.8	30	13.52	
6.15 h	Ac-228			1158	29.9	911.2	
						968.97	
698.60 d	Th-228	5423.15	72.2			84.37	
1.91 a		5340.36	27.2				
3.627 d	Ra-224	5685.37	94.92			240.99	
		5448.6	5.06			292.7	
55.8 s	Rn-220	6288.08	99.886			549.73	
		5748.24	0.114				
0.15 s	Po-216	6778.3	99.9981				
10.64 h	Pb-212			331.3	83.1	238.63	
						300.09	
		6050.78	25.13	2252.1	55.37	727.33	
60.54 min	Bi-212	6089.88	9.75			1620.5	
		5768.03	0.611				
3.06 min	Si α			1796	48.7	2614.53	
	TI-208			1286	24.5	583.19	
0.300 μs	Si β^-	8784.86	100				
	Po-212	10,180	42				

2.2. Activity curves of radionuclides

The decay chain of ^{232}Th is complex, leading to a series of 10 alpha, beta and gamma radiation emitters before reaching a steady state as 218 Pb (Fig. 2). In order to take the contribution of each of the progeny at time t into account, we calculated the activity of each radionuclide as a function of time with Matlab® software. The source, acquired 32 years ago, was hypothesized to be pure, without any decay products. As the number of nuclei was not known at the initial time, it was taken as one mole (6.02×10^{23} atoms). Then, the disintegration of each of the progeny was calculated using its half-life and the Bateman formula:

$$N_n(t) = \lambda_1\lambda_2 \ldots \lambda_{n-1} \cdot N_1(0) \left[\frac{e^{(-\lambda_1 t)}}{(\lambda_2-\lambda_1)(\lambda_3-\lambda_1)\ldots(\lambda_n-\lambda_1)} + \frac{e^{(-\lambda_2 t)}}{(\lambda_1\lambda_2)(\lambda_3-\lambda_2)\ldots(\lambda_n-\lambda_2)} + \ldots + \frac{e^{(-\lambda_n t)}}{(\lambda_1-\lambda_n)(\lambda_2-\lambda_n)\ldots(\lambda_{n-1}-\lambda_n)} \right]$$

with λ_i the half-life of nuclide i produced in the chain of decays ($i \in [1, n]$).

By varying the time from t_0, the date of acquisition of the source, to the present - a duration of 32 years, we obtained activity-time curves for each radionuclide. The ratio of activity of each daughter compared to the parent nucleus was calculated in order to determine the proportions of each of the progeny at $t = 32$ years. These data were used to draw up the source file in the GATE code to model the ^{232}Th.