

Quality of Life and Management of Living Resources

Key Action 4 – Environment and Health

THz-BRIDGE

Tera-Hertz radiation in Biological Research, Investigations on Diagnostics and study on potential Genotoxic Effects

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Deliverable D-6

Assessment of radiation effects on human lymphocytes (MN assay)

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INTRODUCTION

Genotoxicity and effects on cell proliferation in human peripheral blood lymphocytes following THz radiation were investigated by applying the cytokinesis-block micronucleus assay. For this purpose whole blood samples from 9 healthy donors were employed and exposed for 20 minutes to 1 mW average power Free Electron Laser radiation in the frequency range 120-140 GHz.

MATERIALS AND METHODS

Irradiation procedure

The irradiation experiments have been carried out at the Compact FEL Facility operating at the ENEA Research Centre in Frascati. It utilizes a microtron as electron beam source at energies between 2.3 and 5 MeV [1]. A permanent magnet undulator with 8 periods of 2.5 cm has used to generate coherent radiation in the frequency range between 90 and 150 GHz and a specific THz Delivery System (TDS) has been designed to irradiate whole blood samples, as described in details in Deliverable 1.

2 ml whole blood were irradiated from the top of the Petri dish. In presence of blood sedimentation, lymphocytes settle about half way through the sample thickness, with the red blood cells laying underneath and the serum above.

Two different FEL frequencies have been tested: 120 and 130 GHz, as reported in Table 1. The THz radiation has been monitored by a calibrated pyroelectric detector that measures the transmitted power and allows to calculate the total energy delivered in a given irradiation time taking into account the macropulse repetition frequency.

Frequency	Bunch duration	Bunch interval	Pulse duration	Pulse rep. rate	Average power	Irradiation time	Delivered energy
120 GHz	50 ps	330 ps	4 μ s	2 Hz	1 mW	20 min.	1.20 J
130 GHz	50 ps	330 ps	4 μ s	2 Hz	0.6 mW	20 min.	0.72 J

Table 1 – Irradiation parameters

An important consideration concerning the study of the irradiation of THz radiation in biological systems, and more specifically in whole blood, is that, at these frequencies, the macroscopic structure of the sample components must be taken into account for a proper modelling of the interaction. In fact, due to the surface tension of the liquid samples, a “meniscus” effect is observed because the liquid “sticks” to the side surfaces of the polystyrene container, thus reducing the effective thickness of the sample at the centre of the container, where the transmission is measured. As a result we can say that the lymphocytes that are placed in the centre of the Petri dish experience a higher value of the incident electric field and power, respect to those that are close to the wall, not only because the radiation intensity is higher, but also because the serum thickness is smaller and thus the absorption too. The values of the incident power on the blood surface and of the electric field on the lymphocytes are summarised in Table 2.

	$\langle P \rangle^{120GHz}$	\hat{P}_{4ms}^{120GHz}	\hat{P}_{50ps}^{120GHz}	$\langle P \rangle^{130GHz}$	\hat{P}_{4ms}^{130GHz}	\hat{P}_{50ps}^{130GHz}
Power impinging on the sample	1 mW	125 W	825 W	0.6 mW	75 W	495 W
E on lymphocytes	8.3 V/m	2.9 kV/m	7.6 kV/m	6.4 V/m	2.3 kV/m	5.8 kV/m

Table 2: Calculation of the Electric field on the lymphocytes.

Biological procedure

Blood samples from 9 healthy subjects aged between 30 and 45 years (mean age: 39.33 ± 4.82 years) were collected at ENEA Occupational Health Unit. From each blood sample two Petri dishes were set-up containing 2 ml each: one to be exposed, the other to be sham exposed. From each of them a lymphocyte culture was set up; a third culture was also set up as a control. Blood samples from 6 donors were employed to perform experiments at 120 GHz, while blood samples from the remaining 3 donors were employed for exposures at 130 GHz.

Lymphocytes cultures were set-up by adding 0.8 ml whole blood to 9.2 ml of RPMI medium supplemented with 15 % heat-inactivated foetal calf serum, 2 mM L-Glutamine and 100 μ l of Phytohemagglutinin as mitogen.

In order to block cytokinesis, 44 hours after PHA stimulation, Cytochalasin-B (2 mg/ml in DMSO) was added to lymphocyte cultures to give a final concentration of 6 μ g/ml. 72 h after PHA stimulation, cells were collected and processed for slide preparation, as described in Deliverable 1. Coded slides were scored blindly and MN were counted in binucleated cytokinesis-blocked cells with a light microscope at 1250 x magnification following standard criteria. For each subject/treatment 1000 binucleated cells with well preserved cytoplasm were examined from various slides.

On the same slides cell proliferation was also evaluated on 500 cells by means of the cytokinesis-block proliferation index (CBPI) [2].

In order to compare each exposed sample with its own control, two tailed paired Student's *t* test was applied to compare unexposed and exposed cultures as far as MN frequency and CBPI are concerned. Differences were considered to be significant at $p < 0.05$.

RESULTS

The effect of 20 minutes exposure on blood samples from 9 healthy subjects, 6 exposed to 120 GHz and 3 exposed to 130 GHz, are depicted in figure 1 and 2 for micronucleus frequency and cell cycle kinetics, respectively. The results obtained are reported for control, sham-exposed and exposed cultures. It appears that the environmental conditions at FEL laboratory do not influence the MN induction at both 120 and 130 GHz, as shown by comparing control and sham exposed cultures ($p=0.380$ and $p=0.260$ for 120 and 130 GHz respectively). Performing the same comparison, cell cycle kinetics also results unaffected ($p=0.724$ and $p=0.500$ for 120 and 130 GHz respectively).

Concerning the induction of genotoxic effects, the results obtained indicate that such radiation, in the experimental conditions adopted, does not affect micronucleus formation, as shown by comparing sham exposed cultures with exposed ones, at both frequencies employed ($p=0.182$ and $p=0.43$ for 120 and 130 GHz, respectively). The same comparison doesn't show any effect also in terms of CBPI ($p=0.133$ and $p=0.50$ for 120 and 130 GHz, respectively).

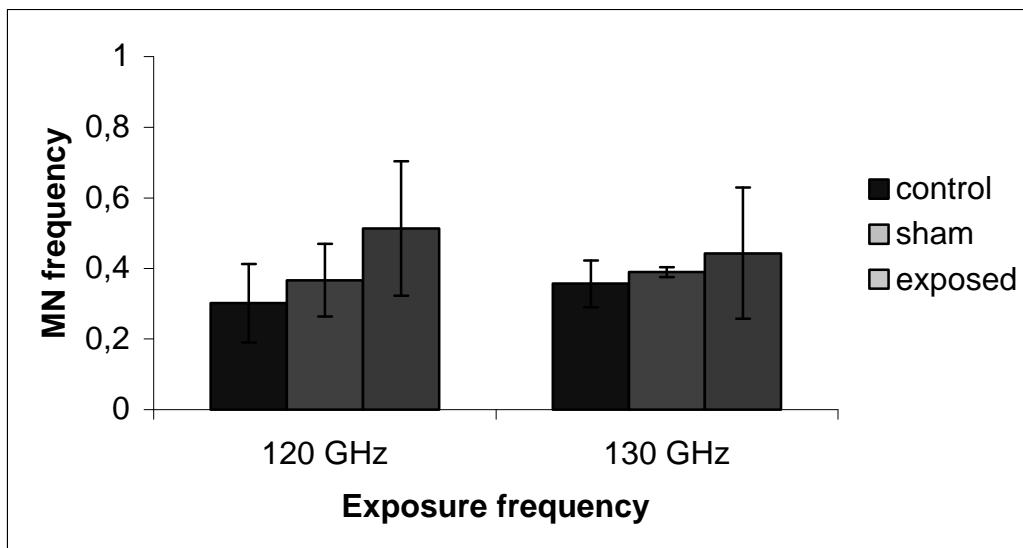


Figure 1 – Micronucleus frequency in control, sham exposed and exposed human lymphocyte cultures. Data are reported for 120 GHz (6 subjects) and 130 GHz (3 subjects) exposure frequency.

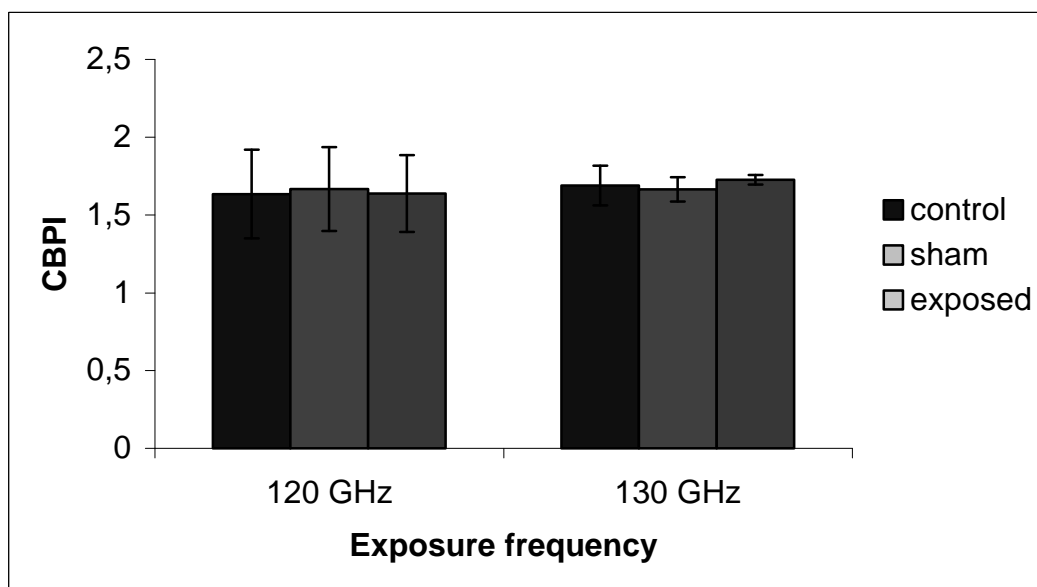


Figure 2 – Cytokinesis block proliferation index in control, sham exposed and exposed human lymphocyte cultures. Data are reported for 120 GHz (6 subjects) and 130 GHz (3 subjects) exposure frequency.

DISCUSSION

In this study the potential health hazard induced by electromagnetic radiation in the THz region, the portion of electromagnetic spectrum laying between 100 GHz and 20 THz at the boundary between the microwave and the infrared regions, has been considered. Despite the recent technological applications of THz radiation in biology and biomedicine, which are based on the specific spectroscopic fingerprints of biological matter in this spectral region, very little is known about its interaction with biological systems. Genotoxic effects are one of the most interesting aspects in the

risk assessment of human exposure to ionising and non-ionising electromagnetic radiation, due to the close correlation between DNA damage and cancer occurrence [3]. Since lymphocytes are a well-known biological system playing a key role in the defence mechanisms and are easily obtainable from peripheral blood, they have been largely used as a biological model for studying the potential genotoxic effects of such radiation. Among the cytogenetic techniques allowing to test DNA damage at cellular and molecular level induced by various chemical and physical agents, the cytokinesis-block micronucleus(MN) technique is a very sensitive and simple indicator of chromosome damage, which also provides information on cell cycle progression [4-6].

The cytokinesis-block MN technique has been employed in this study to investigate the genotoxic effects on human peripheral blood lymphocytes exposed in Go phase for 20 minutes to 1mW average power Free Electron Laser (FEL) radiation at two frequency values of 120 and 130 GHz respectively.

The results presented here are particularly interesting since, to our knowledge, this is the first time that the THz radiation is investigated as potential genotoxic agent by applying the MN technique. The limited number of subjects considered, at least in the case of 130 GHz, does not yet allow definitive conclusions. Ongoing studies on a larger number of individuals should provide further, more complete information on the topic.

References

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