Effects of mm-waves on human fibroblasts in-vitro

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***Abstract*— A comprehensive study of the in-vitro exposure of human fibroblasts to millimeter waves in a wide spectral region is under way. The evaluation of different biological endpoints as result of exposure to pulsed radiation in the frequency range 100 - 150 GHz with the ENEA Compact FEL is reported. A new exposure set-up for irradiation to CW radiation in the range 18-40 GHz will also be presented.**

I. Introduction

In this study we address cito-genotoxic effects on human fibroblasts as in-vitro cellular model exposed to millimeter wave radiation. Fibroblasts are the most common cells of connective tissue in animals. They are a type of cell that synthesize the extracellular matrix and collagen and play a critical role in wound healing.

II. 100 - 150 GHz exposure set-up

The radiation source used for exposure in this frequency range is the ENEA Compact Free Electron Laser [1,2]. The time structure of the radiation is composed by pulses 4 μs long, with a maximum repetition frequency of 10 Hz. Each pulse is composed by a train of micro-pulses, each 50 ps long, with 330 ps spacing. Such a low duty cycle allows the investigation of the effects of high peak power, while maintaining a low average power, typically a few mW, incident on the sample, thus avoiding heating effects.

A radiation launching device was designed and built for such frequencies in order to provide the necessary expansion to irradiate from below the 5 cm diameter polystyrene Petri dish, containing a monolayer of fibroblasts cells. Sham-exposed samples were placed on the same working plate with the THz exposed samples. During the irradiation experiments the temperature of the sample was monitored by means of an infrared camera FLIR A325.

III. Biological endpoints and results

Various biological endpoints have been addressed: analysis of cytogenetic markers (Micronucleus assay, Comet assay, Chromosome Non-disjunction, telomere length), cell cycle kinetics, protein expression and ultra-structural observations of cells.

In most of the assays no differences were observed between the exposed samples and the control ones, but a significant increase of micronuclei (MN) was observed in irradiated samples, and the MN induction evaluated by CREST indicated that THz radiation could induce chromosome loss [3].

Ultra-structural analysis also showed radiation-induced effects, but such an evidence disappears in samples observed 48h after irradiation, demonstrating that morphological changes revealed immediately after exposure were transitory.

IV. 18-40 GHz setup

A new CW exposure layout has been set up in order to expose samples to lower frequencies. The source is a YIG oscillator, delivering 20 mW output power in the frequency range between 18 and 40 GHz. The layout of the setup is sketched in fig. 1.

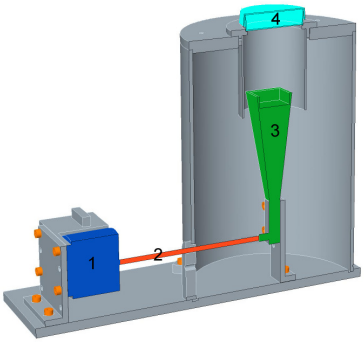

Fig. 1. Layout of the 18-40 GHz exposure set-up. 1) source; 2) transmission line; 3) horn antenna; 4) Petri dish

The mm-wave radiation is launched using a horn antenna and the size of the beam naturally expands by diffraction up to the 5.2 cm diameter of the Petri dish. The emission frequency for irradiation was chosen to be 25.28 GHz.

A picture of the experimental setup is visible in Fig. 2. A collecting horn and a detector are placed over the sample to verify the radiation transmission through the sample itself.

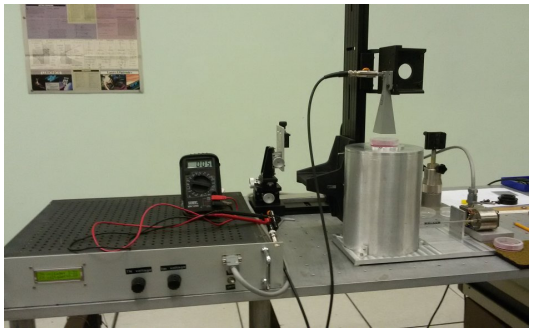

Fig. 2. Layout of the 18-40 GHz exposure set-up. 1) source; 2) transmission line; 3) horn antenna; 4) Petri dish

The system has been set up in order to obtain a radiation beam profile as uniform as possible on the sample. The intensity profile of the THz beam at the sample position has been measured by means of a point detector placed on an automated 2D scanning system. The result is shown in Fig. 3.

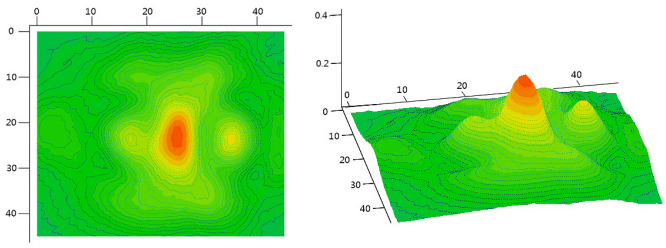

Fig. 3. Intensity profile of the beam measured at the sample position for an emission frequency of 25.28 GHz.

V. BIOLOGICAL ENDPOINTS AND PRELIMINARY RESULTS

Various biological endpoints are being addressed: analysis of cytogenetic markers (Micronucleus assay, Comet assay, Chromosome Non-disjunction, γ-H2AX quantification and Telomere length analysis). Here are reported the preliminary results about Micronucleus assay and γ-H2AX quantification.

A conventional MN test was performed using the Cytokinesis Block Micronucleus (CBMN) technique that permits the analysis of MN in binucleated (BN) cells [4]. In Tab. 1 the results of the induction of MN in HFFF2 cells after exposure to MW radiation from two independent scorers are reported. A significant increase of MN in irradiated cells compared to control cells was observed for both scorer (χ^2<0.01) with a mean of 11 MN in 1000 BN in control samples and 21 MN in 1000 BN cells in the irradiated samples.

Tab. 1. Micronuclei induction after MW treatment of HFFF2 cells.

HFFF 2	Scorer	Control (MN in 1000 BN)	Treated (MN in 1000 BN)	χ^2
I Exp	1	11	19	*p<0.01*
	2	11	23	*p<0.01*
Mean		11	21	*p<0.01*

To better understand if MW exposure is able to induce direct DNA damage onto HFFF2 cells we have performed the immunofluorescence analysis of phosphorylated histone H2AX foci 30 min, 2 h and 24 h after treatment (Fig. 4). No differences were observed between irradiated and control cells at each time after exposure (p>0.05).

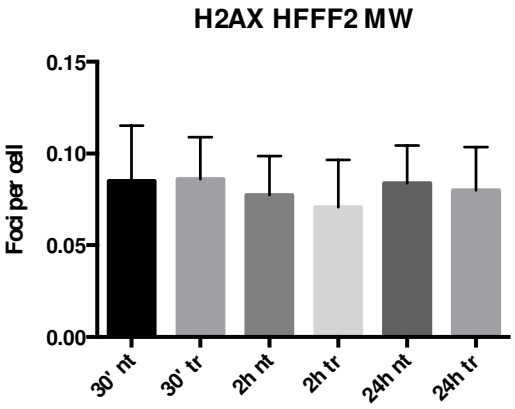

Fig. 4. γ-H2AX foci analysis. No differences were observed 30 min, 2 h and 24 h after treatment with MW radiation respect to control cells. nt: not treated; tr: treated.

ACKNOWLEDGMENTS

The authors gratefully acknowledge funding from the Italian Ministry of Defense - GREAM Project.

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