Studying the effect of high-power coherent terahertz pulses on mesenchymal stem cells

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Abstract. Question of safety of THz radiation for living objects (starting at the cellular level and ending with the organism in whole) is still a matter of controversy and requires further study. In this paper we present experimental results of studying the effect of terahertz laser pulses on monolayer cell culture. Bone marrow mesenchymal stem cells are exposed to multiple (from 50 to 4200) THz pulses with intensities of about 10 MV/cm. No short-term effect is observed 24 h after cell irradiation.

1. Introduction

Despite the rapidly increasing application of THz radiation in fundamental research and many practical areas (medicine, security and military applications etc), the question of THz radiation safety for living objects (ranging from single biomolecules and cells to whole organisms) is still a matter of controversy and requires further study. Considerable efforts have been directed towards studying the interaction of THz radiation with biological systems within the framework of the international project THz-BRIDGE (2001–2004). In the official conclusion, the possible genotoxicity of THz radiation (genotoxic effect was detected in lymphocytes) as well as change in the permeability of liposome membranes under certain conditions was noted. Although genotoxicity of THz radiation was confirmed, it was not clear under which conditions such effects may arise.

Quite a few studies of bioeffects of THz radiation have been performed since the THz-Bridge project completion [1]. Terahertz radiation effects on a diverse range of cellular processes including cellular death and membrane permeability [2,3], gene expression alteration [4,5] and even deoxyribonucleic acid (DNA) double-strand breaks [6,7] have been investigated. The latter is considered to be one of the most dangerous types of DNA damage, since it can lead to cell death or cancer. Several studies have been conducted in recent years regarding THz radiation effects on animals. Survival ability and the lifespan of the fruit flies [8] as well as behavioural changes in mice [9] and initiation of wound-like response in mouse skin [10] under the action of THz radiation have been evaluated.

There are two main factors that influence the interaction of THz radiation with biological systems: the composition and optical properties of the biological systems to be irradiated (index
of refraction, absorption coefficient, etc) and THz exposure parameters (frequency, exposure time, power density etc). It should be noted that the THz sources with relatively low output power of $\sim 100 \mu W$ have been applied in the majority of investigations published up to 2012 [11]. The development of intense sources of THz radiation has considerable potential for studies of THz radiation interaction with biological tissues and may provide in-depth understanding of the mechanisms of interaction.

In this paper, we report on the application of extremely intense THz pulses for irradiation of living cells. We exposed bone marrow mesenchymal stem cells (MSC) to a broadband THz radiation and the effect of the THz radiation on the cell viability was evaluated 24 h after THz exposure.

2. Experimental setup

The experimental setup scheme is shown in figure 1(a). Generation of THz radiation is carried out by optical rectification of femtosecond pulses of infrared laser radiation (wavelength is 1240 nm, pulse duration is 100 fs) in the nonlinear organic DSTMS crystal [12, 13]. Pump laser facility generates laser pulses with energy of 20 mJ and a repetition rate of 10 Hz. Diameter of the DSTMS crystal is 5 mm.

Thus, laser beam is partially cut by iris diaphragm to obtain a flat-top intensity profile across the beam and optimal laser fluence of 10 mJ/cm$^2$. The efficiency of conversion of laser radiation in THz is about 1.5%. A pulse of THz radiation with a duration of about 0.7 ps is generated, with a spectrum width of 0.1–4.5 THz. The energy of THz pulse is about 75 $\mu$J.

The spectrum of THz pulse is shown in figure 1(b). It should be noted that the main energy of Thz pulse is contained between 1 and 2.5 THz frequencies. Petri dish with test cells is placed at a linear motorized stage. Terahertz radiation is focused to $\approx 260 \mu$m spot size (at $1/e^2$ level) by 2-inch off-axis parabolic mirror and is directed through the bottom of the plastic dish. Estimated field strength is about 10 MV/cm.

Our preliminary results show that polystyrene is transparent in 0.1–4.5 THz frequency range so that terahertz pulses reach freely cell culture monolayer at the bottom of the dish.
3. Methods

3.1. Cell culture
Monolayer culture of bone marrow mesenchymal stem cells (MSC) was chosen as a test object. Cells were cultured under standard conditions (5% CO$_2$, 37°C) in complete growth medium consisting of DMEM/F12 (1 : 1, Biolot, Russia) supplemented with gentamicin (50 µg/ml; Paneco, Russia) and FBS (10%; HyClone, United States). The medium was changed every 2–3 days and cells were cultured to confluent monolayer. At the third passage at 90% confluence, cells were harvested using versene solution (Biolot, Russia) and 0.25% trypsin (Biolot, Russia) solution and transferred to 35 mm Petri dish at cell density $10^5$ cells per dish. The dishes that reached 90–100% confluence were divided into 3 groups—the experimental group that was irradiated with THz pulses and two control groups: intact control group was kept under standard culture conditions and the parallel control group was kept at room temperature for the same time as the experimental cultures.

3.2. Cell irradiation
The experimental dishes were placed at the linear stage and marked by 2 lines that denoted trajectory of THz beam at the beginning and at the end, respectively. Then we chose several points along the line trajectory to effect by THz pulses. Relative coordinates of those points were registered.

Two different irradiation regimes were used. Mode A implied constant movement of the dish with velocity of 5 µm/s for about 7 min. This means that each point along the trajectory of THz beam has been irradiated by about 50 terahertz pulses. In mode B the dish was kept still and irradiated with THz pulses for 7 min that resulted in about 4200 of pulses in total. In general, a total time, the cells were kept outside the incubator, did not exceed 25 min. After that, the dishes were placed back to incubator and cell viability was assessed 24 h after the THz exposure.

3.3. Cell viability assay
Detection of the impact of THz pulses was carried out by staining cell cultures in all 3 groups with vital fluorescent dyes. Sodium bis-benzimide (Hoechst 33258, Fluka), which interacted with DNA minor groove, was used to stain all nuclei. Propidium Iodide (PI), which stained dead cells, was used for the primary rapid assessment of cell viability. Cells in the area of THz impact and in equal area in control plates were studied using phase contrast (Axiovert 40 CFL, Zeiss) and fluorescence (PALM Combisystem, Zeiss) microscopy; the ratio of PI stained cells to the total number of cells was evaluated for each experimental and control plate.

4. Results and discussion
Terahertz pulses with electric field strength of about 10 MV/cm in the focal plane of focusing parabola were applied to study the effect on MSC culture for the first time. Subsequent staining with PI showed no positively stained cells in both areas A (irradiated in mode A) and B (irradiated in mode B). Figure 2 demonstrates the pictures obtained for phase contrast microscopy 24 h after the experiment. No morphological differences were found between irradiated cells (neither in mode A, nor in mode B) and both parallel and intact control groups 24 h after irradiation. The dead cells were also absent in all studied groups. These results indicate that the chosen irradiation parameters did not cause any short-term harmful effect on MSC cultures.

There have been nearly 50 publications on biological effects of THz radiation over several decades. However, the subject still remains highly controversial. The majority of studies found no or very slight effects caused by terahertz radiation. However, the number of investigations, containing the evidence that THz radiation influences living biological objects, has grown.
significantly during the last decade. One possible explanation for this trend is the development of high-power THz sources and their applications to biological systems. The benefit of using such sources (combining high peak power with low average power) is the possibility of studying the effects of THz radiation on living cells with minimal risk of thermal effects induction.

Several recent experimental studies have demonstrated important results on possible bioeffects of THz radiation. The probability for control of gene expression in mouse mesenchymal stem cells by application of THz radiation has been discussed in [14]. Important genetic alterations after \textit{in vivo} THz irradiation of mouse skin has been identified in [10]. As stated in [7], the exposure of artificial skin tissue to intense picosecond THz pulses may induce double strand breaks in DNA. At the same time, it was observed that DNA damage repair mechanisms were also activated under the action of THz radiation. Authors used pulsed broadband radiation (1.7 ps pulse duration, 1 kHz repetition rate) with peak incident THz electric fields at the sample in the range of 70–220 kV/cm. The exposure to intense THz pulses for 10 min was enough to cause a significant induction of H2AX phosphorylation indicative of DNA double strand breaks.

Our newly developed THz source provides generation of THz laser pulses with peak electric fields of $\sim 10$ MV/cm that is more than an order of magnitude higher as compared to recent published data (220 kV/cm for optical rectification THz source [7] or electric field of 1.7 kV/cm for NovoFEL experimental setup [6]). Thus, the aim of this study was to investigate the short-term effects of extremely intensive THz pulses on the viability of mesenchymal stem cells. We supposed that the utilization of such a high-power THz source would provide unambiguous information about bioeffects of THz radiation and allow us to determine the safe levels of exposure to THz radiation. Moreover, we believed that using high-power THz source may enable us to decrease significantly the exposure time typically required for effects to occur and, thus, to simplify the process of cell preparation and handling during the experiment (outside a controlled environment of the incubator).

Here we report that the exposure to a broadband high-power THz radiation does not lead to any morphological changes or compromise the viability of the MSCs in monolayer cell culture during the first 24 h after irradiation. However, several considerations that may explain this observation should be taken into account. The first one is the type of the model cells employed in our experiments. As it has been discussed in [3], there may be several types of cells potentially not sensitive to THz radiation. Thus, for example, the lack of cytotoxic effect of the external THz field was observed in cells from epithelial and supporting tissues, as well as in populations of stem cells. Moreover, the dependence of the THz effect has been recently shown not only
on irradiation parameters of THz source, but also on the degree of stem cell differentiation prior to irradiation [15]. The third consideration is that despite huge field strength applied, pulse duration of 0.7 ps may be insufficient to damage the cell enough to cause its death since the repetition rate and the mean power of terahertz radiation are low. It is also possible that total exposure time is not enough even for strengths of about 10 MV/cm. And last but not least one is the methods employed for estimation of cytotoxic, genotoxic and any other adverse effects. Here we analyze only the morphology of exposed cells and their viability by staining with fluorescent dyes. It has been shown [5] that such type of analysis may sometimes be effective in revealing some general differences between exposed and control cells, for example, accumulation of lipid-like droplets in cells can be monitored by light microscopy. However, this is not always the case and more complicated and sensitive methods are usually required to find and thoroughly investigate the effects of THz radiation. Thus, for example, cell viability assay showed no significant differences in exposed human keratinocytes as compared to sham-exposed cells in [16] whereas analysis of transcriptional response of cells to THz radiation displayed numerous over- and under-expressed genes in comparison to sham-exposed cells.

Thus, here we report the preliminary results on studying the short-term effect of THz pulses of extreme field strength on MSC monolayer culture. Further investigations involving more detailed analysis with assessment not only cellular viability, but also their functional activity and changes in gene expression, optimization of THz radiation parameters (for example, increasing the exposure time) as well as employment of different cell types as a model are required to obtain reproducible and reliable results on bioeffects of intense THz radiation.

5. Conclusions
In this study mesenchymal stem cells were used as a cellular model for investigation of bioeffects of intense THz radiation. We exposed MSC monolayer culture to high-power THz pulses with peak electric field of \(\sim 10\) MV/cm at two different irradiation modes. Cell viability analysis performed in 24 h after irradiation by fluorescent staining with propidium iodide and Hoechst dyes did not reveal increase in the number of dead cells as compared to non-exposed cells. No significant morphological changes were observed in THz-exposed cells when compared with control groups.

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