University of Pecs

Doctoral School of Physics

Laser physics, nonlinear optics, and spectroscopy Program

The Effect of Intense Terahertz Pulses on *Eisenia Andrei* Earthworm Tail Regeneration

Ph.D. Thesis

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1. BACKGROUND AND OBJECTIVES

Terahertz Science and technology features a big selection of applications, and sometimes it's the potential for an application that has driven the innovative research during this field. Applications in THz will be largely classified into three areas (although there's overlap between these categories)– THz imaging, THz spectroscopy, and THz communication [1].

The investigation biological effects of THz radiation on biomolecules like DNA, peptides, lipids, and certain cells like fibroblasts, lymphocytes, and epidermal cells was the focus of several studies, resulting in contradictory experimental results. However, there is a consensus that THz waves can modify the activity of biomolecules or cells by either thermal effects or non-thermal biological effects. The former one proved to be harmful generating cell death (apoptosis, necrosis) [2],[3] or morphological injury of neurons in vitro experiments [4], and expression of stress molecules (heat shock proteins, and DNA damage markers) in irradiated cells [5].

From a biological point of view, in addition to these opposite results, there is some experimental evidence on the neutral effects of THz radiation in biological systems. Applying lower frequency (0.14 THz) or power (0.45 J/cm^2) no effect of THz radiation was observed on the differentiation, cell viability of human keratinocytes and neurons [6], [7]. It was also found that THz radiation had no significant effect on the morphology, adhesion, proliferation, and differentiation of certain human cells, like epithelial and embryonic stem cells further on the chromosomes of the lymphocytes in vitro experiments [8], however, THz radiation can mediate the growth and proliferation of certain cells under definite exposure conditions [9]. It influences the plasma membrane permeability resulting in either suppression or facilitation of neuronal activity [10], [11], the natural dynamics of DNA, and thereby influences intricate molecular processes involved in gene expression and DNA replication [10], and it accelerates cell differentiation and cellular reprogramming [12].

Based on these studies, it seems that THz radiation can have a strong and versatile influence on biological systems, which sensitively depends on the irradiation parameters (spectral distribution, power, duration, electric field strength) and the morphological and/or physiological characteristics of the biological targets [12]. There is a relatively simple in *vivo* experimental method, used from the 18th century, the regeneration of the surgically ablated tail segments of earthworms. Earthworms have an enormous capacity to regenerate lost tail segments [13], and this process is mediated by some internal and external factors. The neural dependence of the regeneration was promulgated by Morgan [14], The effects of the internal factors on cell proliferation, differentiation, and tissue morphogenesis can be modified by certain environmental conditions, such as temperature [15], osmotic stress [16], and electromagnetic waves like laser [17].

The regenerating capacity of earthworm after cut effected by several factors, while applying a regular experimental protocol permits more chances to grasp the important effects from physical parameters, Terahertz pulses induce segmental renewing via cell proliferation overriding the endogenous regeneration program of the earthworm *Eisenia andrei* [18]. Therefore THz pulses provide a proper opportunity to research the biological effects on cellular level redifferentiation, proliferation, and differentiation.

The physical results can contribute to the clarify the THz pulses absorption, scattering, and transmission on the biological tissue level. Moreover, the transmission of THz pulses through an organ (body wall) and the whole postclitellar segments of the earthworm *Eisenia andrei* are studied. The power transmission results for THz pulses are compared to those for near-infrared (1030 nm), and green (515 nm) pulses. Simple calculations indicate that THz pulses of 5 μJ energy cause only a small temperature increase of about 0.26 °C, suggesting that heat effects may be less important in biological effectiveness. The applied 0.3–0.6 THz frequency range to the cultured cells was weak enough that any thermal effects could be ignored [9].

My study aimed to investigate the effects of high-intensity THz pulses on the standardized posterior segment regeneration of the earthworm *E. andrei*. Segment regeneration has certain discrete phases constituting the post-extirpation repairing process. Another goal was to demonstrate the interaction mechanism of THz pulses, and optical pulses with biological materials, by measure the power of THz pulses transmits through the sample. And compare the absorption coefficients of THz pulses with green light, and near-IR transmitted in isolated tissues, in the equal optical- tissue interaction circumstances such as water content in the tissues, tissues structures, laser power, and irradiation time.

2. METHODS

1- The effect of THz pulses on segment regeneration kinetics of excised *Eisenia andrei* earthworms is investigated, by cut 5 caudal segments then irradiated for 5 minutes by single-cycle THz pulses (5 µJ energy, 0.3 THz mean frequency, and 1 kHz) were generated by optical rectification of short infrared laser pulses in $LiNbO_3$ crystal using the conventional tilted-pulse-front technique [19], compared also on the same conditions for irradiated animals by green light (515 nm) and non-irradiated (control- sham) earthworm. The number of renewal segments on each animal group has been counted after two, three, and four weeks as illustrated in (Fig. 1).

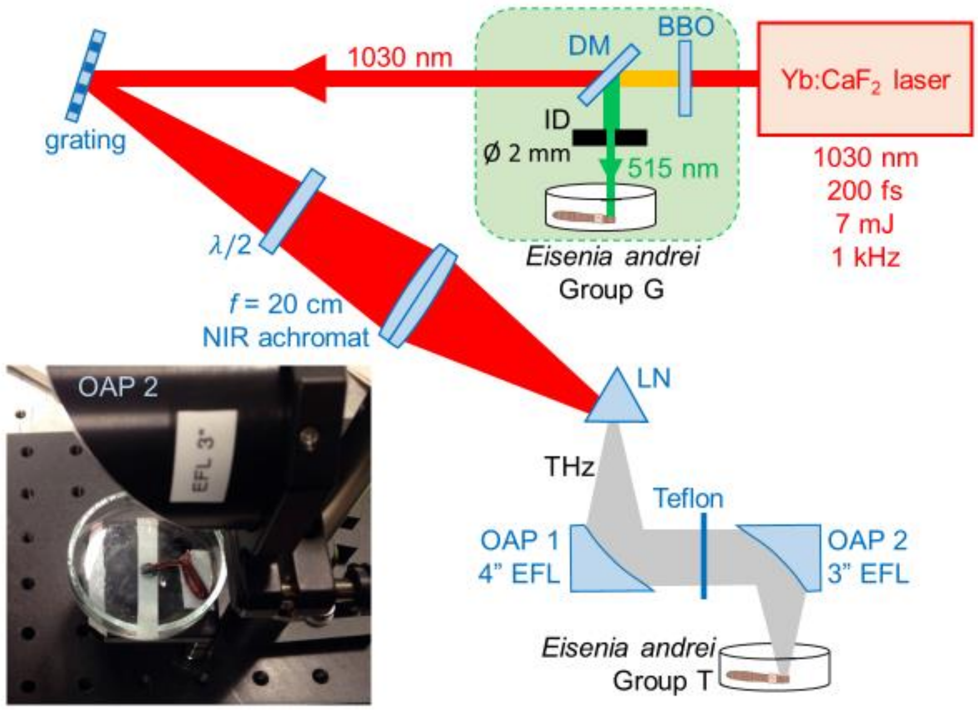

Fig. 1. Scheme of the irradiation setup. The *E. andrei* specimens of Group G were placed into the green laser beam. The specimens of Group T were placed into the focus of the THz beam. In this case the components within the green box were removed from the setup. DM: dichroic mirror, λ/2: half-wave retardation plate, OAP: off-axis parabolic mirror, EFL: effective focal length. The bottom left inset shows a photograph of an anaesthetized earthworm at the THz irradiation position.

2- Following to irradiation experiments 5 animals from each group of the regenerating body parts with 3 or 4 original segments were dissected and fixed in freshly prepared formalin-acetic acid solution, then investigated histologically by a Nikon Optiphot-2 photomicroscope. Moreover, statistical analyses have been carried out by one-way (ANOVA), and Student's t-test to calculate the p-value in the different irradiated animal groups.

3- The experimental animals, adult (clitellated) specimens of the earthworms *Eisenia andrei* (Annelida, Oligochaeta, Lumbricidae) were hand-sorted from our standard laboratory breeding stocks[20]. Selected animals were kept on paper wadding wetted with tap water for three days at room temperature. Before the surgical interventions, the animals were anaesthetised by saturated carbonated water then from some specimens of different sizes either the dorsal part of the body wall, or ten-twelve postclitellar segments were dissected.

4- The transmitted power measurements were carried out for the green 515 nm, and IR 1030 nm pulses laser of central wavelengths, 180 fs pulse duration, up to 1 mJ pulse energy, and controlled intensity were delivered to position IR by an Yb:KGW regenerative amplifier operating at 1 kHz repetition rate. Before the sample, an iris diaphragm reduced the beam size to about 2 mm, a diameter smaller than the sample size. Green laser pulses of 515 nm wavelength were produced by the phase-matched second-harmonic generation of the infrared laser pulses in a BBO crystal. Also, THz transmission measurements were carried out by a commercial time-domain THz spectrometer (TDTS) of model Tera K8 manufactured by Menlo Systems. Nearly-single-cycle THz pulses were generated in the TDTS with a biased photoconductive antenna illuminated by femtosecond laser pulses. The THz pulses were collimated and focused on the sample by a pair of TPX lenses. The peak of the amplitude spectrum of the broadband THz pulses was at about 0.65 THz. For the investigated strongly absorbing tissue samples, the useful spectral range was extending from about 0.2 THz to 2.0 THz.

5- A steady-state heat equation was used to model the heating effect of radiation on the tissue and to compare it to pure water. The beam was assumed to propagate into the z-direction. In order to the simplicity, temperature variation only in z-direction was considered. Such an approximation is suitable for the body wall tissue, where the thickness of the tissue ($\leq$0.33 mm) is nearly one order of magnitude smaller than its lateral size

(≥2.5 mm) and the typical size of a focused THz beam (≥2 mm for frequencies of 0.3–0.5 THz).

3. NEW SCIENTIFIC ACHIEVEMENTS

Applications of THz radiation have been developed recently in particular imaging and spectroscopy, furthermore, high-intensity THz pulses have been generated by the department of physics at the university of pecs. These reasons motivated me to investigate the effects of THz pulses on a specific animal model (*Eisenia Andrie*) earthworm we used in our study. That animal has shown several changes on cells and tissues by THz pulses, in the following statements:

I. This finding is the first experiment proved that single-cycle THz pulses of 5 µJ energy, 0.30 THz mean frequency, and 1 kHz repetition rate induced the cells proliferation (indicated by the high number of mitotic cells) is presented in (Fig. 2c), and both histogenesis and organogenesis producing a significantly higher number of regenerated segments of earthworms. The most noticeable alterations in THz-treated animals were the more intense development of the new central nervous system and blood vessels is shown in (Fig. 2b). These results were demonstrated that THz pulses are capable to efficiently excite biological processes and suggest potential applications in medicine **[S1].**

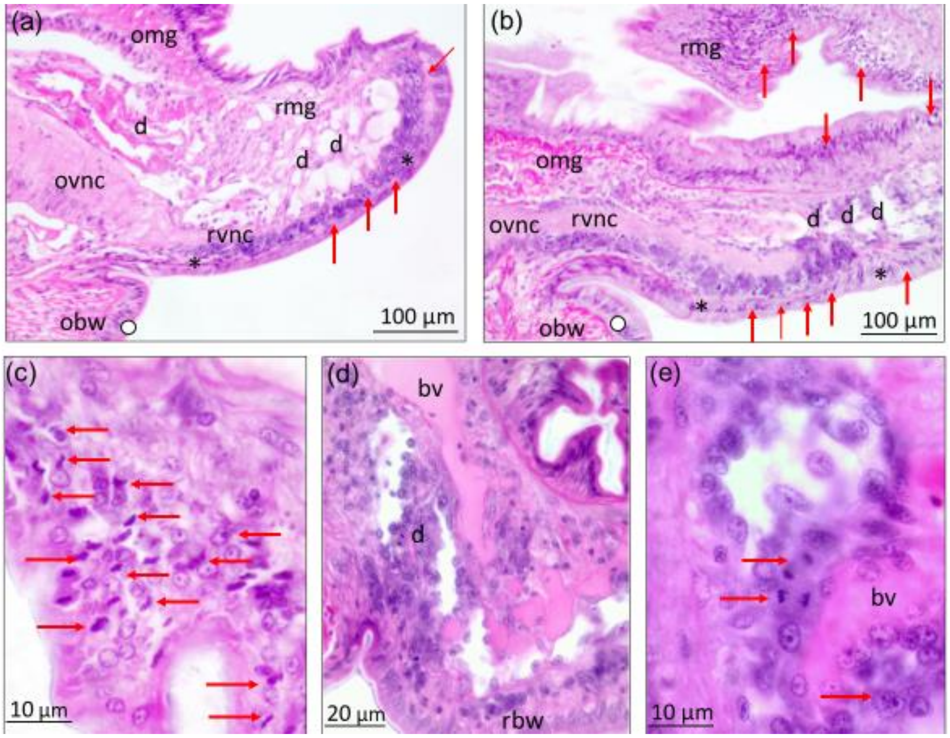

Figure 2. Histological characteristics of the regenerated segments of control (a), and THz-irradiated earthworms (b-e) on the 14th postoperative day. Since group G animals are characterized by the same histological alterations than group C ones their sections are not presented in the figure. Abbreviations: ovnc: old ventral nerve cord, rvnc: regenerated ventral nerve cord, obw: old body wall, rbw: regenerated body wall, d: dissepiment, omg: old midgut muscular tissue rmg: regenerated midgut muscular tissue, bv: blood vessel, arrows: mitotic cells, circles: old epithelial body layer, stars: new epithelial layer.

II. This study demonstrated various tissue types such as midgut epithelial, ventral nerve cord, blood vessels, and muscular tissue have been developed rapidly after exposure to THz pulses for 5 minutes. On the other hand, this study showed no effects of the green light wavelength on regenerated segments (Fig. 3)., and the low number of blastema cells accumulated towards tissues after the wound, on specific investigated time **[S1].**

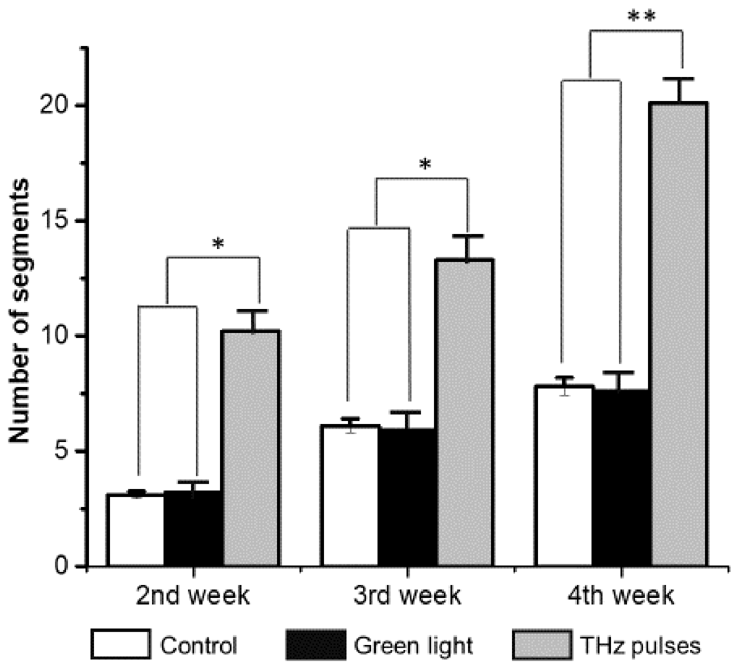

Figure 3. Kinetics of the segment regeneration in control (sham-exposed) and green light or THz irradiated *E. andrei* earthworms. Data represent mean ± SEM (n=45, *: $p < 0.05$, **: $p < 0.01$).

III. It has been experimentally revealed the transmitted power for the green optical pulses followed the Beer-Lambert law of exponential attenuation for all thicknesses and tissue structures, near-infrared IR pulses were significantly deviating from this, which hints at the dependence on tissue structure. Whereas, the absorption coefficient (126 cm^{-1}) of THz pulses in tissue structures was dozens of times higher than the absorption coefficient of IR, and green lights consecutively in tissues is presented in (Fig. 4).

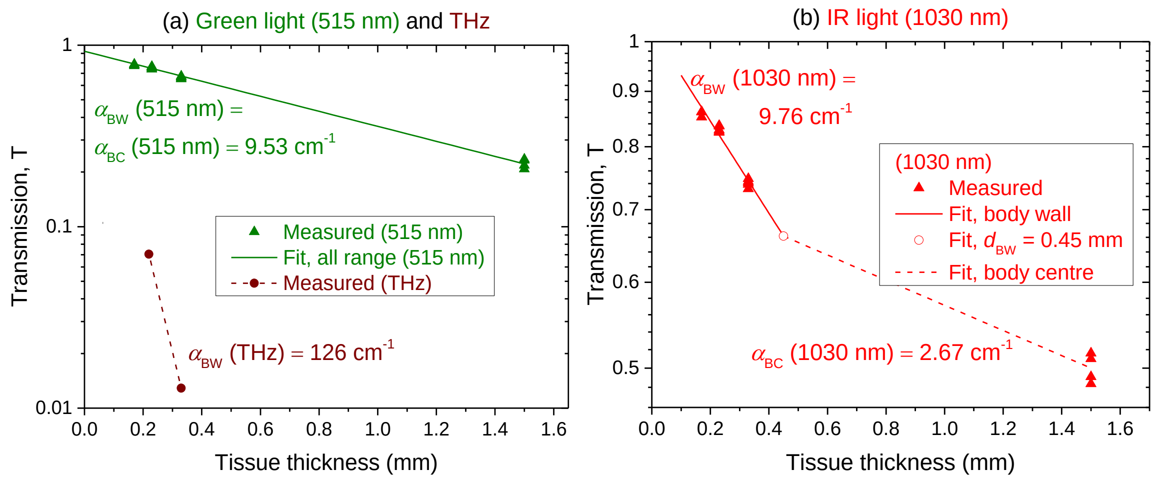

Figure 4. (a) Measured power transmission for green light and THz radiation as function of the sample tissue thickness. (b) Measured power transmission for infrared light as function of the sample tissue thickness. The lines corresponding to green, and infrared indicate exponential fit curves. The dashed line in case of the THz data is a guide to the eye. The empty symbol in (b) indicates the body-wall fit extrapolated to 0.45 mm thickness.

IV. The THz absorption coefficient by tissues was determined by time-domain THz spectroscopy measurements within frequency range from 0.2 THz to 2.5 THz. The absorption varies from 80 cm^{-1} at 0.2 THz to 273 cm^{-1} at 2.5 THz. Furthermore, the knowledge of tissue absorption properties is an important prerequisite to estimate the biological effectiveness of radiation. The intense pulsed THz radiation can have a profound biological impact. Despite small penetration depths of about 0.1 mm for the 0.2 THz to 2.5 THz range considered in our work, global (nonthermal) biological effects can occur **[S2].**

V. It has been numerically determined the small amount of THz pulses 5 μJ energy and 1 kHz repetition rate (5 mW average power), less than 0.41 K of THz pulses heat has transferred to the certain tissue thickness by using a simple calculation model, suggesting that heat effects may be less important in biological effectiveness. This can be drastically different for mJ-level THz pulse energies, which have become available recently **[S2].**

4. ARTICLES RELATED TO THE TOPIC OF THIS THESIS

- **M. Abufadda *et al.*,** “Terahertz pulses induce segment renewal via cell proliferation and differentiation overriding the endogenous regeneration program of the earthworm Eisenia andrei,” *Biomed. Opt. Express*, vol. 12, no. 4, pp. 1947–1961, 2021, doi: 10.1364/boe.416158 [18].

- **M. H. Abufadda *et al.*,** “Absorption of Pulsed Terahertz and Optical Radiation in Earthworm Tissue and its Heating Effect,” *J. Infrared, Millimeter, Terahertz Waves*, 2021 [21].

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