THz spectroscopy of whole blood, plasma and cells in mice of SHR line with various pathology

A Panchenko$^{1,2,3}$, M Tyndyk$^{1,2}$, O Smolyanskaya$^1$, M Sulatskiy$^1$, O Kravtsenyuk$^1$, N Balbekin$^1$, M Khodzitsky$^1$

$^1$ ITMO University, 197101, Kadetskaya 3, Saint-Petersburg, Russia
$^2$ N N Petrov Research institute of oncology of Russian Ministry of Health, 197758 Leningradskaya str., 68, Pesochny, Saint-Petersburg, Russia
$^3$ Federal Almazov North-West Medical Research Centre of Russian Ministry of Health, 197341, Akkuratova str., 2, Saint-Petersburg, Russia

E-mail: o_smolyanskaya@mail.ru

Abstract. This paper is devoted to studying of optical properties of whole blood and blood plasma in SHR mice grafted Ehrlich’s carcinoma and mice with chronic inflammation at the terahertz frequency range. Additionally physiological saline solution suspension of ascites Ehrlich’s carcinoma cells was explored.

1. Introduction
Over the last few years an opportunity of using pulsed terahertz (THz) sources for medical biomedicine practice including low-intensity systems has become extensively investigated [1-5]. Pulsed terahertz radiation has a great potential for medical diagnostics, it allows analyzing a broad spectral range of the investigated object within a short scan period of time. Special properties of terahertz radiation are exhibited in its broad frequency range and low intensity.

2. Materials and methods
2.1 Animals
Outbred Swiss-derived (9 male and 36 female) SHR mice (NN Petrov Research Institute of Oncology) three month of age (25-30 g) were used. Animals were kept in the ventilated vivarium in the cages of T2 type during 12 hours of light and 12 hours of darkness at 22 $\pm$ 2 °C, they received laboratory chow (Laboratorkorm, Moscow, Russia) and tap water ad libitum.

2.2. Tumor model
Ascitic Ehrlich’s carcinoma of mice (NN Petrov Research Institute of Oncology) was used. Mice were subcutaneously inoculated with suspension of tumor cells ($10^6$ cells per 0.2 ml of 0.9 % NaCl solution, Dalchimpharm, Russia) for induction of solid tumors. Tumor growth was estimated, the day of tumor grafting was accounted as “0”: the tumor volume was calculated by ellipsoid formula:

$$V = \frac{ab^2}{2},$$

where $a$ is a length, and $b$ is a width of tumor node.
2.3 Inflammation model
The model of a chronic inflammation in mice was used [6]. 12-O-tetradecanoylphorbol 13-acetate, TPA (Sigma, USA) was applied (20 μl 0.01% weight/volume in acetone) on each ear (10 μl per each side of an ear) at day 0, 2, 4, 6 and 8. Control animals were treated with the same volume of acetone (analytical grade, Ecos, Russia). The ear swelling was estimated.

2.4 Blood samples
Blood samples were obtained from a tail cut into K₂EDTA MiniCollect tubes (Greiner Bio-One). Blood counts were performed (Mindray 2800Vet, China) prior to Terahertz spectroscopy. Blood plasma samples were prepared from blood obtained during the decapitation procedure into K₂EDTA MiniCollect tubes and centrifuged (5 minutes at 1500 rpm) after visual control of clotting.

2.5 Experimental procedures, distribution of animals in groups
Experimental procedure consists of three set:
1. whole blood measurements in intact 9 male (group 1) and 4 female (group 2) mice;
2. whole blood measurements in female mice with subcutaneous Ehrlich’s carcinoma and with inflammation: 4 mice injected physiological solution at day 0 (group 3); 4 mice with Ehrlich's carcinoma (group 4); 4 mice with Ehrlich's carcinoma and treated by cyclophosphamide intraperitoneally 48 hours after tumor grafting (200 mg/kg, Baxter Oncology, Germany) (group 5); 5 mice with inflammation (group 6); 5 mice with acetone treatment (group 7, control to group 6);
3. blood plasma and tumor cells suspension measurements in 4 control mice (group 8) and 6 mice with ascites Ehrlich's carcinoma (group 9).

2.6 Terahertz time-domain spectrometer
The study was performed by time-domain spectroscopy using a system developed in ITMO University [7]. Characteristics of the emitted THz radiation are the following: the frequency ranges from 0.1 to 1.8 THz, average power of 0.3 μW and pulse duration of 2.7 ps. Most of the power was spread within the 0.1 to 0.6 THz frequency range. In the frequency range from 1.0 to 1.8 THz the signal did not exceed 1% of the maximum value. The sample was fixed perpendicularly to the optical axis in the focal plane using a two-axis motorized translation stage. Spectral resolution during measurements was about 15 GHz in the reflection mode. System control was performed by an in-house written code in LabVIEW environment.

2.7 Statistical analysis of results
Experimental results were statistically processed using the Student's test (MS Excel 2010, Origin Pro 2015 programs). Distinction was proposed as reliable at p < 0.05.

3. Results and discussion
The refractive and absorption indices (Fig. 1) of whole blood for males and females coincide in THz frequency of 0.3 - 0.9 THz. The differences in blood count were not found (data not shown).
The second set results are presented on Fig. 2 and Fig. 3. The typical tumor growth was observed in mice with Ehrlich's carcinoma (group 4, mean tumor volume at the 14-th day was $1.56 \pm 0.32 \text{ cm}^3$) and this growth was inhibited after cyclophosphamide injection (group 5, tumor volume at the 14-th day was $0.19 \pm 0.14$, $p < 0.01$). Refractive and absorption indices of whole blood are shown on Figure 2. It is obvious that optical properties of whole blood in $(0.3 \div 0.9) \text{ THz}$ frequency range for control mice (group 3), mice grafted by Ehrlich's carcinoma (group 4) and mice with Ehrlich's carcinoma injected by cyclophosphamide (group 5) practically coincide. The difference was found only in absorption coefficient of whole blood in mice of group 5 on 14-th day. The comparison of group 5 with day “0” and with group 3 isn’t shown any differences in blood count. Moreover the recovery of white blood cells decreased by cyclophosphamide injection on the 7-th day was observed on 14-th day. So, leucopenia does not influence whole blood refractive and absorption indices at the THz frequency range. The difference found in group 5 whole blood absorption coefficient on 14-th day cannot be explained by changes in hematological parameters.

Whole blood optical properties (refractive and absorption indices) at terahertz frequency range were also assessed at 10-th day of inflammation in mice. The obvious oedema and inflammation reaction were observed after TPA application (group 6) and they increased during the experiment (mean ear thickness at day 0 was $0.30 \pm 0.02$ mm for right ear and $0.30 \pm 0.00$ mm for left ear; at day 10 these values were $0.62 \pm 0.19$ mm, $p < 0.05$ and $0.56 \pm 0.13$ mm, $p < 0.05$ accordingly). Blood count at day 10 revealed granulocytosis: $4.10 \pm 0.57$ (control) and $6.80 \pm 1.77$ (inflammation), $p < 0.05$. Differences in optical characteristics (absorption and refractive indices) of whole blood in mice with inflammation and corresponding control group as well as with Ehrlich's carcinoma bearing mice were not found.

Absence of influence of white blood changes on optical properties can be caused by huge amount of erythrocytes whose absorption and refractive indices are proportional to their concentration [8].
Figure 2. Refractive (a) and absorption (b) indices of whole blood in the terahertz region on SHR mice (days 0, 7 and 14 after grafting). Control group is group 3.

Figure 3 Refractive (left) and absorption (right) indices of whole blood in the terahertz region on SHR mice (days 0, 7 and 14 after grafting). (c, d) Control group 4: tt – mice grafted by Ehrlich's carcinoma. (e, f) Control group 4: tc - mice with Ehrlich's carcinoma injected by cyclophosphamide.
The third set of experiments was done to evaluate blood plasma and suspension of tumor cells of Ehrlich's carcinoma in physiological saline solution (Fig. 4). The refractive and absorption indices of tumor cells (Ehrlich's carcinoma) suspension in THz frequency range did not depend on their concentration in solution. The difference between blood plasma and tumor cells suspension is not significant in term of refractive indices. But the difference between plasma and tumor cells suspension absorption indices was found for all investigated frequency ranges (0.3 ÷ 0.9 THz). Differences in optical properties of blood plasma in group 8 and group 9 were found at the frequencies 0.7 and 0.8 THz. The values of absorption and scattering indices of samples were grown with water concentration. This observation is consistent with the results of the study Jeong et al. [8].

![Figure 3](image)

Figure 3. Refractive (left) and absorption (right) indices of object in the THz frequency range on suspension of tumor cells (a,b) and blood plasma (c,d), group 8 and 9.

4. Conclusion
The whole blood optical properties (refractive and absorption indices) do not depend on the growth of Ehrlich's tumor and inflammation process. Changes in white blood cells characteristics do not affect the optical properties of whole blood at THz spectral range. The optical properties of biological systems at THz spectral range are highly dependent on the water concentration in the system.

Acknowledgments
This work was supported by the Government of Russian Federation (Grant 074 – U01).
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