The study of spectral changes in THz range in normal and pathological skin in vivo depending on the dehydration methods used

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ABSTRACT

The terahertz (THz) attenuated total reflectance (ATR) imaging of normal and pathological skin under the action of various dehydration agents was carried out in vivo. Studies were conducted on animal models (the mouse), patients with diabetes, and healthy volunteers. For measurements, each animal was leaned against the ATR prism of the skin surface, and several locations in the skin of each animal were analyzed. Places on the skin for analysis were chosen so that the intensity spectra of the THz signal were practically the same for selected points. THz spectra measurements were carried out every 10 minutes within 45 minutes interval under the action of a dehydration agent. 40% glucose was shown to provide the most effective improving tissue optical clearing effect in the THz range.

Keyword list: THz spectroscopy, diabetic tissues, clearing agents

INTRODUCTION

The changes associated with diabetes mellitus can affect various organs, including the skin. With the development of diabetes mellitus, the skin becomes susceptible to various infections, the skin fibrosis is observed, the skin becomes more dry and vulnerable (because of the malfunction of leucocytes), and the patient suffers from skin itch. These symptoms are manifestations of diabetes mellitus complications related to the hyperglycemia and glycation of proteins. Dermatologic manifestations of diabetes mellitus have various health implications. Besides, some of them can be life-threatening. Knowledge of the relation of skin characteristics and diabetes mellitus can provide insight into the present or prior metabolic status of patients. Information about such findings may help in the diagnosis of diabetes; also, it probably can be followed as a marker of glycemic control.[1]The normal content of free glucose (glycemia) in blood amounts to 3.3–5.5 mmol/l (60–100 mg/dl).[2]

The recent development of terahertz (THz) technologies has allowed a fast measuring broadband spectroscopic data in this spectral range from a variety of substances, including human tissues. THz spectroscopy provides visualization of the spatial distribution of absorption spectra of living skin. The use of THz has been shown to be effective in the study of burns,[3,4] cancer [5], and different melanin content.[6]

Tissue-clearing techniques have received great attention for imaging and for the potential to be applied in optical diagnosis.[7] In principle, tissue clearing is achieved by reducing light scattering through a combination of lipid removal, size change, and matching of the refractive index (RI) between the optical clearing agent (OCA) solution and the tissue.

Recently, the efficiency of the optical clearing of skin, kidney, and cornea samples in the terahertz wavelength range was shown to be higher for non-diabetic samples than for the diabetic ones at the application of glycerol solution of different concentration.[8] The diffusion coefficient of glucose is significantly lower in diabetic skin compared to healthy skin. It has been found that the optical clearing of the skin in diabetes is slower compared to healthy skin, the measured glucose diffusion coefficients are lower in the skin in diabetes compared to the control.[9,10]

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In this paper, we are considering the possibility of increasing the quality of the THz imaging of healthy and diabetic skin using tissue optical clearing (TOC) agents based on diabetes small animal model and humans *in-vivo*.

MATERIALS AND METHODS

In this work, laboratory mice (figure 1) with type 1 diabetes were examined, and healthy human skin and the skin of patients (figure 2a) with type 1 diabetes were measured *in vivo* by terahertz time-domain spectroscopy (THz-TDS).

Type 1 diabetes mellitus (DM 1) in C57BL/6 outbred mice was induced by intraperitoneal injection of streptozotocin (STZ) in 0.1 M citrate buffer (pH 4.5) at a dose of 180 mg/kg after fasting for 6 hours.[11] Control animals were treated with an equal volume of 0.1 M citrate buffer (pH 4.5). After three weeks from a day of STZ injections, blood samples were collected by tail-tip amputation. No anesthesia was used at the time of the blood sampling to avoid unequal variations between animals and also avoid the effects of anesthesia on the blood glucose levels. Mice were fixed during blood collection from tail-tip. The FreeStyle optimum (Abbot, UK) glucometer was used for the glucose and ketones measurements.[11]

Three weeks after STZ injections, diabetic mice blood glucose levels were 21.0 (19.6-24.6) mmol/L, and ketones were 1.2 (1.1-1.3) mmol/L (Me(Q1-Q3; n=6). For control animals: 5.2 (4.6-5.5) mmol/L and 0.4 (0.3-0.5) mmol/L, respectively (Me(Q1-Q3; n=6). High blood glucose and ketone levels are strong evidence for successful DM 1 in mice induction.

Control and diabetic mice were anesthetized preliminary by tiletamine and zolazepam (Zoletil®). Then animals were shaved, and TOC agents were placed on the skin surface. The TOC agents were included glycerol, oily alcohol solution, omnipaque, and 40% glucose solution. The diagnosis of diabetes patients was carried out by standard clinical methods.

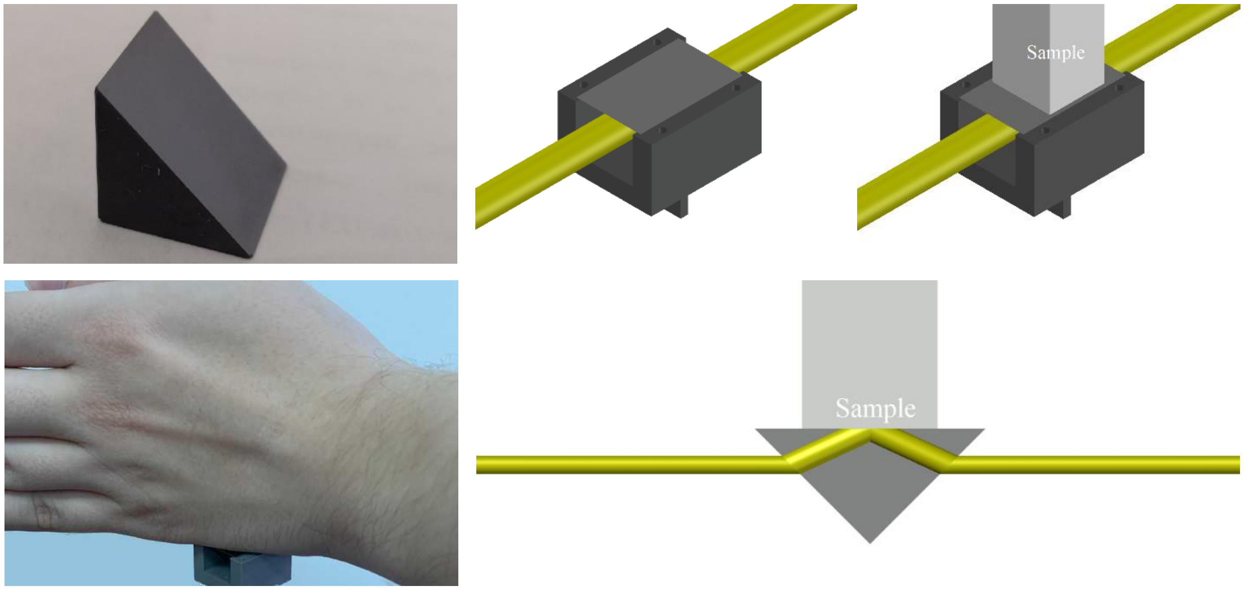
The experimental part of the human's research was carried out according to the principles of Good Clinical Practices. A protocol of the research was approved by the Local Ethics Committee of the Cancer Research Institute of Tomsk National Research Medical Center.

Figure 1. Photo of laboratory mice

The THz absorption spectra of the skin were recorded using an EXPLA T-spec spectrometer using a high-resistance silicon prism in the attenuated total reflectance (ATR) mode (figure 2). The measurements were carried out according to the scheme shown in figure 2b. The ATR phenomenon is accompanied by the penetration of light into an optically less dense medium. The reflection of light under these conditions is described by the Fresnel equations. In the case of weak absorption, the solution of these equations comes down to obtaining an approximate relation for the reflection coefficient in the form:

$$R = \exp(-\alpha N d),$$

where α is the absorption coefficient, N is the number of reflections; d is the effective thickness of the absorbing medium, equivalent in physical meaning to the thickness of the sample in absorption spectroscopy. It allows using an experimental scheme (Figure 2b) to study the absorbing properties of the medium using the standard absorption approach.[12, 13]



a b

Figure 2 - Prism ATR (a), the reflection scheme (b)

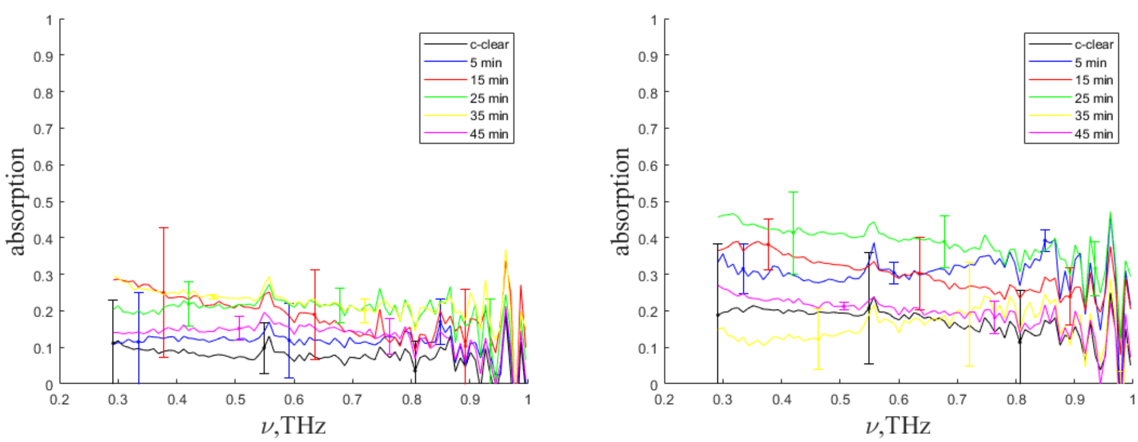
The signal passing through the prism in the absence of a sample was used as a reference one. Then the sample was placed on a prism, and the absorption spectrum was recorded. Temporal averaging (over 256 points) has been made.

The study involved 3 mice with type 1 diabetes and 3 healthy mice. For each mouse, four skin areas were selected, on all of which a TOC agent was placed and kept for 60 minutes. Before each experiment, a recording of the absorption spectrum of the surface of each of the 4 skin areas has been made. Further, the absorption spectrum was recorded 5 minutes after the application of the TOC agent. Each subsequent recording was carried very 10 minutes.

Preliminary studies of the skin of a patient with diabetes and a healthy volunteer have been carried out using a solution of glucose as the TOC agent.

EXPERIMENTAL RESULTS AND DISCUSSION

Figure 3 shows the averaged absorption spectra of the skin before and after the application of various TOC agents for mice with type 1 diabetes and healthy mice.



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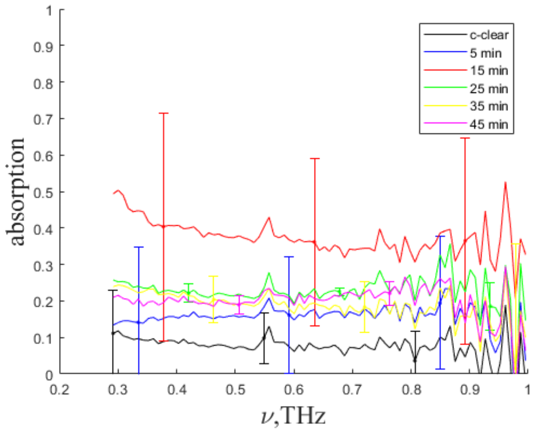

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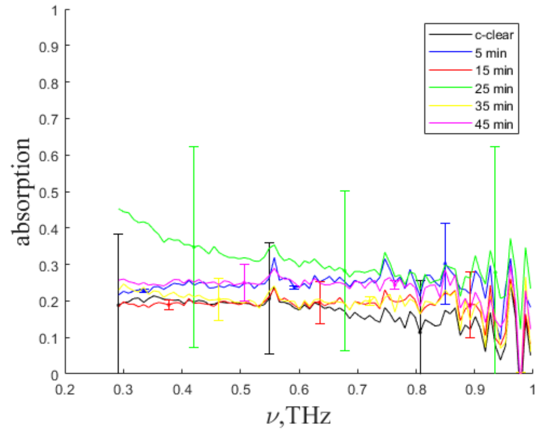

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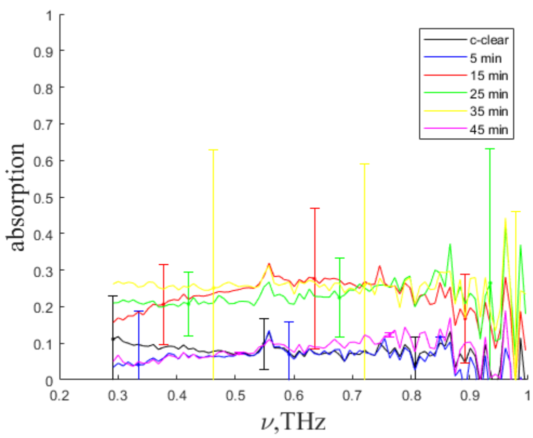

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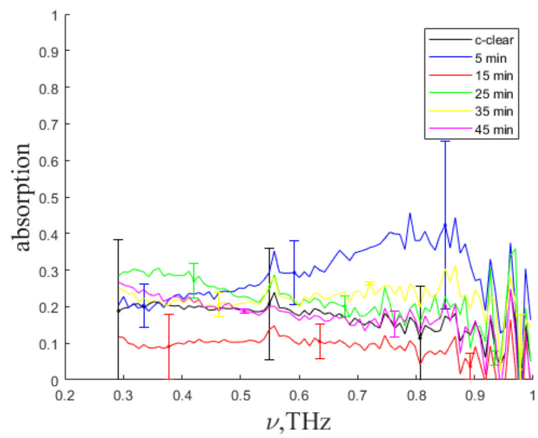

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Figure 3 - Averaged absorption spectra of the skin with various TOC agents for mice with diabetes (a - glycerol, c - oil, e - omnipaque) and healthy mice (b - glycerol, d - oil, f - omnipaque)

Figure 3 shows that for mice with diabetes, upon exposure to the skin with a TOC agent, the absorption increases first (about 15 minutes), then the absorption decreases. However, this decrease in absorption is negligible. For healthy mice, the picture is similar. The most promising clearing agent was 40% glucose solution.

Figure 4 shows the averaged absorption spectra of the skin of patients with type 1 diabetes and healthy volunteers before and after applying a 40% glucose solution. Evidently, the 40% glucose solution is an effective TOC agent for patient with diabetes but almost does not change the absorption characteristics of the skin of a healthy volunteer.

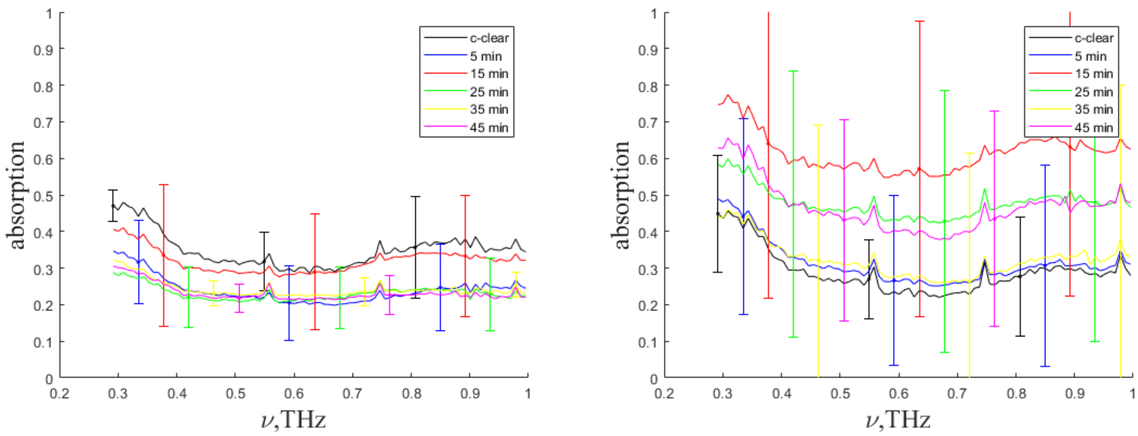

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Figure 4 - Averaged absorption spectra of the skin of patient with type 1 diabetes (a) and healthy volunteer (b) before and after applying a 40% glucose solution as a TOC agent

CONCLUSIONS

In-vivo measurements of healthy and diabetes humans and mice's skin were carried out by THz spectroscopy. It has been shown that 40% glucose solution is promising for use as tissue clearing agents in the THz spectral range for patients with diabetes. In common, the TOC agents allow improving the quality of THz imaging of diabetic tissue.

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