Terahertz Spectroscopy Used to Distinguish Breast Epithelial Cell Lines

Juhyung Kim[1], Elizabath Philip[1], Ursula L. Triantafillu[2], Yonghyun Kim[2], Seongsin M. Kim[1]
[1] Electrical and Computer Engineering, University of Alabama, Tuscaloosa, AL, 35487 USA
[2] Chemical and Biological Engineering, University of Alabama, Tuscaloosa, AL, 35487 USA

***Abstract*— THz spectroscopy has the potential to be used in identifying and characterizing cell types. In this study, normal 184A1 and metastatic MDA-MB-231 breast epithelial cell lines were characterized using transmission mode of THz Time-Domain Spectroscopy as they transitioned from their active to in-active state. A frequency domain scan was performed at set time intervals on both cell lines and the result showed that there is a time domain variation between the scans of the two cell lines that can be used to identify each cell type.**

I. Introduction

RESEARCH utilizing THz spectroscopy has shown promising results in distinguishing various breast tissues such as healthy fibrous breast tissue, healthy adipose tissue, and breast cancer [1,2]. These advancements in THz spectroscopy have even lead to the creation of new and improved intra-operative tools to distinguish breast cancer tumor margins [1]. While there are studies done on distinguishing tumor mass in breast tissues *ex vivo* and even *in vivo* using THz, it is evident that very little research has been done on breast cancer cell lines. Tissues contain a heterogeneous population of cells, and it is difficult to distinguish signals from normal cells from cancerous ones. As a result, the goal here is to study the interactions of THz radiation on cancer cells instead of tissues in order to characterize and identify the unique properties of cancer cells. By monitoring the cells in real-time as they transition from their active (in the presence of the cell culture media) to inactive state (as the media dries out), there may be features in either state that can lead to identifying the cells.

If a cell identification technique using THz can be established, it will be useful to characterize and identify cells at laboratories and research institutes that mass produce cell lines. Real-time monitoring of cell lines can also open a window towards studying the effects of various environments such as drugs, pH, temperature, etc. on them. Furthermore, in Ashworth *et al.*2009 the breast tissues were distinguished by determining their respective refractive indices and absorption coefficients, however, the cause for this uniqueness in their optical properties is not understood [1]. Studying the cell lines could also unveil the reason behind the changes in the optical property of the tissue samples.

II. Experimental Procedure

The breast tissue cell line 184A1 (ATCC, Manassas, VA) was cultured in Mammary Epithelial Basal Medium (MEBM) (Lonza) supplemented with 1% penicillin/streptomycin (Corning, Manassas, VA), 0.005mg/mL transferrin and 1 ng/mL cholera toxin. The breast cancer cell line MDA-MB-231 (ATCC) was cultured in phenol-red free Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Grand Island, NY) supplemented with 1% L-glutamine (Life Technologies, Carlsbad, CA) and 10% Fetal Bovine Serum (FBS) (Gibco). Both cell lines were cultured using the recommended protocol by ATCC in Poly D-Lysine 35mm dishes (Benton Dickinson, Bedford, MA) as shown in Fig. 1.

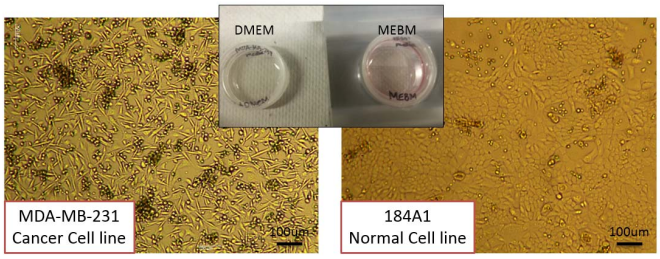

Fig. 1. Microscopic Images of the (a) MDA-MB-231 Cancer cells in the growth media DMEM and (b) 184A1 Normal cells in the growth media MEBM

THz scan was performed over each sample under the transmission mode of THz-TDS. As shown in Fig. 2, a 3D-printed custom holder was used to hold the sample-grown dish and to tilt it at an angle of 90 degrees. The 90-degree tilting was performed to drain out the media because the media, as a liquid, absorbs most of THz radiation.

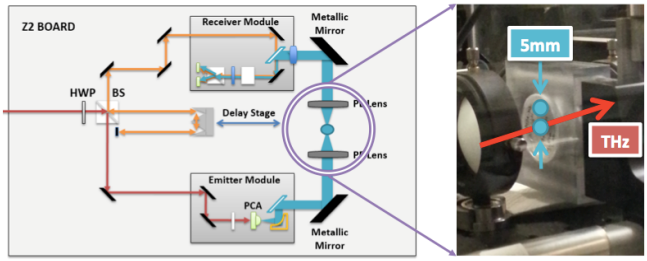

Fig. 2. Schematic diagram of THz TDS and mounted sample holder with cells cultured patria dish.

Both measurements were conducted in purged condition. It was purged with nitrogen gas (N2) during the scan to minimize any absorption due to the moisture since multiple water vapor absorption lines exist in the spectral region around 1.09 THz and 1.72 THz. Poly D-Lysine 35mm dishes without cells measured in advance as a reference. The measurement was performed in two phases; without sealing and sealing the dishes. The first measurement was performed without sealing, so most of the media leaked out. After that another measurement was performed with sealing to verify the difference between spectroscopic response and affection of sealing the dishes. The dish was sealed using a moisture-resistant thermoplastic.

In order to monitor the real-time changes in the sample, the scans were repeated every 8 minutes. This was done twice on each sample to normalize the result; first, at the center of the dish and then at 5mm off the center. Lastly, scans were performed on the negative controls which are the growth

media used for the respective cell cultures. The negative control helped to rule out any peaks stimulated by the media itself.

III. Results

The transmission ratios for each of the scans were plotted by dividing the results from the samples by the results from the reference (sample holder by itself). The plots showed that though the negative controls absorb considerable amount of THz signal there are no distinguishable peaks as shown in the bottom of Fig. 4, implying that the transmission ratios of the cell cultures are solely due to cell-cell or cell-medium interactions.

Exploring the frequency domain was also performed with two phases depending on the sealing the dishes. In both cases, similar peaks were seen, and the peaks disappeared with time due to drying out of the media. Both cell lines displayed similar peaks, and a clear time variation was seen between them as shown in Fig. 3 and 4. Measurement performed without sealant film around the dishes induced to be hardly dried so that there was no any moisture inside of the dish after drained out of the media. Fig. 3 shows the spectroscopic result of both cancer cell and normal cell lines with different time passes. In this case, noticeable distinction was not observed, even though there are some changes with times in both samples.

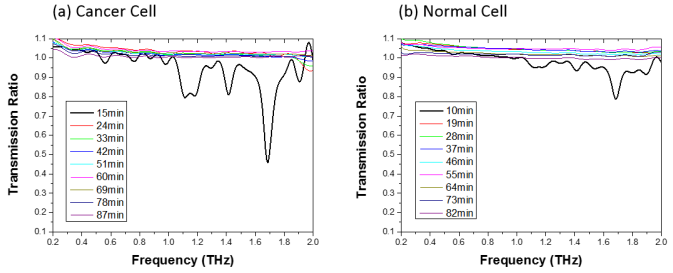

Fig. 3. Measurement result of (a) cancer cells and (b) normal cells in purged and non-sealed condition.

The second phase of measurement with sealing the dishes provided an interesting result as shown in Fig. 4. By sealing the dish, the humidity inside of the dish could be constant during the measurement. A time variation still could be observed clearly, and most of all, there was a distinct peak that differentiated the normal cell from cancer cell around 0.4 ~ 0.45THz. Upon further investigation of the molecular level interactions, we should be able to decipher the reason for this variation and ascertain a method to identify the two cell types. The spectrum for both of the cancer cell and normal cell at 9 minutes showed that the cancer cell peaks disappear in a similar speed with respect to the normal cells. In addition, somehow the transmission ratio has a characteristic such that it has a tendency to have a symmetric peak with the course of time.

Additional measurement performed with dead normal cell lines verified the fact that there is a characteristic such that the transmission ratio graph becomes horizontal straight line along the x-axis when the cells are dead unlike living cells that have a slope as shown in Fig. 5.

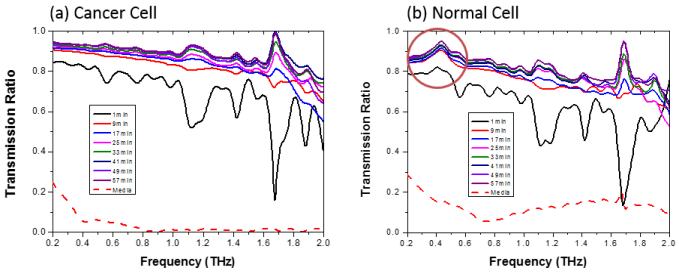

Fig. 4. Real time measurement result of (a) cancer cellsand (b) normal cells in purged and sealed condition.

Dead cells were obtained by leaving them in the room temperature for one week so that they could die due to the lack of nutrition. The reason the slope disappears in the dead cells is directly related to the way we executed this measurement. When we tilt the dish in 90 degrees so that the media was pooled the down by gravity, the living cells have more tendency to hold and to contain the media as a natural metabolism. On the other hand, the dead cells have no ability to react to the changing circumstances. Thus, the final parallel straight lines which represent 100% transmission ratio along the x-axis in Fig. 5 could be clarified as dead cells.

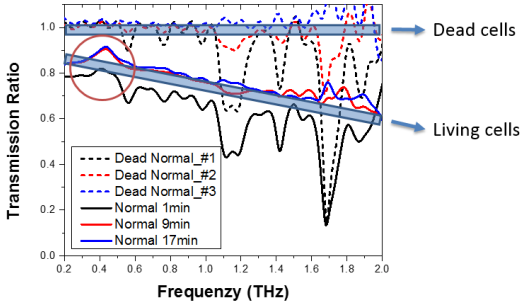

Fig. 5. Difference in transmission ratio of living cells and dead cells using THz-TDS spectroscopy.

IV. Summary

We investigated non-cancerous 184A1 and metastatic cancer MDA-MB-231 breast epithelial cell lines with transmission THz Time-Domain Spectroscopy. The measurement condition affects significantly the spectroscopy measurement results, since THz interaction is rather complicate. We observed unique peak from the normal cell around 0.4THz in the frequency domain compared to cancer cells, which can be used as a fingerprint to distinguish cancer cells from normal cells. Furthermore, additional measurement targeted for the dead cells also displayed clear difference with the living cells in terms of transmission ratio along the THz frequency. Understandably, the further measurement will be needed to further verify the reason and principle of this phenomenon.

References

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