Detection of Proteins Associated with Alzheimer's Disease using a Terahertz Chemical Microscope

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***Abstract*— In recent years, the number of Alzheimer's Disease (AD) has been increasing. We proposed a Terahertz Chemical Microscope (TCM) for early diagnosis of cognitive decline by measuring the concentration of Apolipoprotein AI (ApoA1) and Complement component 3 (C3) in solution as biomarkers for AD using the TCM. As a results, ApoA1 and C3 with the concentration of 0.1 µg/ml and 1.0 µg/ml could be respectively detected by measuring the change in the amplitude of terahertz waves by the TCM.**

Keywords- Terahertz; TCM; Alzheimer's Disease; mild cognitive impairment.

I. INTRODUCTION

In recent years, the number of Alzheimer's Disease (AD) has been increasing as the increase of average life expectancy. Generally, a cognitive function of patients of AD gradually declines. Patients at mild cognitive impairment (MCI) stage, where is the initial stage of AD, have potential to recover by appropriate prevention and treatment. So, early detection and diagnosis of AD is important. Conventionally, imaging systems such as computed tomography (CT), magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT) are used to diagnose. However, these types of modalities are expensive so that diagnostic fees are relatively high. Therefore, primary screening method are essential for early diagnosis of AD.

Practically, detection of concentration of biomarkers, transthyretin (TTR), Apolipoprotein AI (ApoA1) and Complement component 3 (C3), in the blood are measured for the screening [1]. This screening method require multiple testing methods and a large amount of specimens to detect proteins.

In our group, a Terahertz Chemical Microscope (TCM) has been proposed and developed to detect proteins or sugar chains in small amount of liquids [2][3]. In this study, the concentrations of ApoA1 and C3 in solution were measured using the TCM.

Section II describes a schematic of the TCM and experimental procedure. Section III describes the results, and Section IV describes the conclusion.

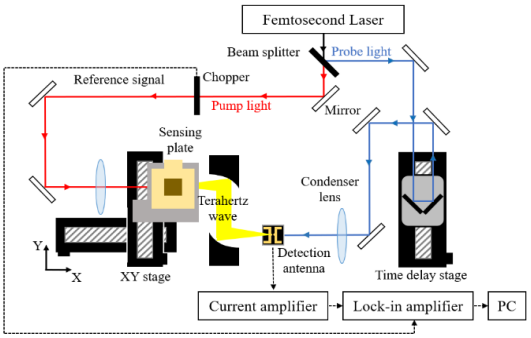

Figure 1. Schematic of the optical system of the TCM.

II. EXPERIMENTAL

The TCM visualize chemical reactions on a sensing plate, which consists of a SiO_2 film and a Si film on a sapphire substrate (Al_2O_3). The thicknesses of the films were a few nm for the SiO_2 film, 500 nm for Si, and 500 µm for the sapphire substrate (Al_2O_3), respectively. When a femtosecond laser is irradiated from the sapphire substrate side to the Si film, terahertz wave is generated in the Si film. The amplitude of terahertz wave depends on the surface potential of Si, which depends on chemical reactions on the sensing plate. Thus, the chemical reaction can be related to the amplitude of terahertz wave radiated from the sensing plate.

Figure 1 shows a schematic of the optical system of the TCM. The femtosecond laser was divided into a pump light and a probe light by a beam splitter, and the pump light was focused onto the substrate side of the sensing plate by an objective lens. The amplitude map of the terahertz wave from the sensing plate was obtained by changing the position of the femtosecond laser across the surface of the sensing plate by moving the XY stage and measuring the amplitude by a photoconductive antenna. The repetition rate of the laser was 82 MHz. And the output power, the center wavelength, and the pulse width of the laser were, respectively, 780 mW, 780 nm, and 100 fs.

Figure 2 shows the protocol to immobilize protein antibodies on the sensing plate to selectively detect antigens. First, the SiO_2 side of the sensing plate was chemically

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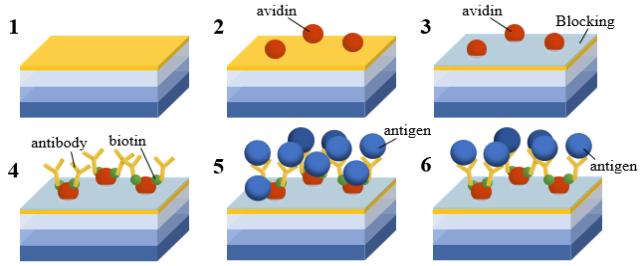

Figure 2. Immobilization protein antibodies and proteins.

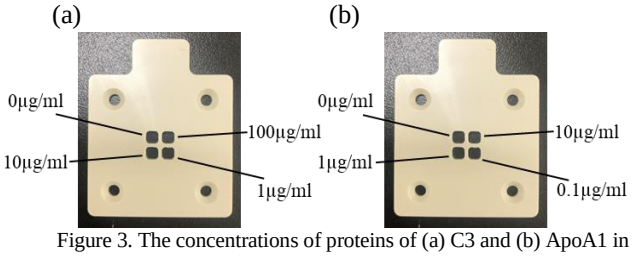

Figure 3. The concentrations of proteins of (a) C3 and (b) ApoA1 in the wells fabricated on the sensing plate.

modified and activated. Then, an avidin was conjugated to the surface of the sensing plate for 24 hours at 4℃. Then, the sensing plate was coated by skim milk to prevent from non-specific reactions on the surface of the sensing plate. After coating, biotin-labeled antibodies were immobilized using avidin-biotin reaction. A human complement C3 antibody (Rabbit, Polyclonal, Biotin conjugate) and Apolipoprotein A1 antibody (Goat, Polyclonal, Biotin conjugate) were respectively used as antibodies for selective detection of C3 and ApoA1. Before measuring the C3 and ApoA1, the amplitude map of the terahertz wave was recorded as a background signal of the TCM. After reacting with C3 and ApoA1, the sensing plate was washed 10 times to remove unbounded antigen. Then, the amplitude map of the terahertz wave was measured to evaluate the change in the amplitude from the background signal.

Figure 3 shows the photos of the solution wells fabricated on the sensing plates and the concentration of (a) C3 and (b) ApoA1 in the wells fabricated on the sensing plate. The volume of each well was 30 μl.

III. RESULTS

Figure 4 shows change in the amplitude of the terahertz waves (a) before and (b) after the reaction of C3 and C3 antibody. The amplitude of the terahertz wave was reduced by the reaction of C3. On the other hand, Figure 5 shows the change in the amplitude of the terahertz waves before and after the reaction of ApoA1 and ApoA1 antibody. The amplitude of terahertz wave increased by the reaction of ApoA1.

The difference between (a) and (b) in Figure 4 and 5 was calculated and then, the values in the each well were averaged. Then, the averaged values were plotted in Figure 6 (a) and (b).

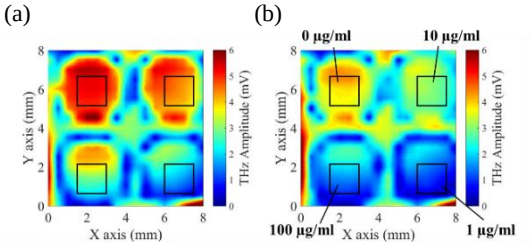

Figure 4. The terahertz maps (a) before and (b) after reaction of C3. The concentrations in the image is the concentration of C3.

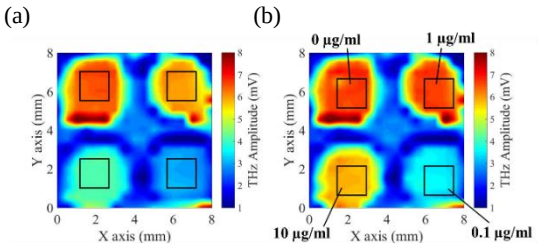

Figure 5. The terahertz maps (a) before and (b) after reaction of ApoA1. The concentrations in the image is the concentration of ApoA1.

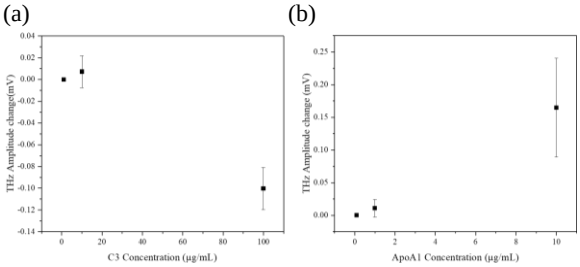

Figure 6. Protein concentration and terahertz wave amplitude of (a) C3 and (b) ApoA1.

One can see that the amplitudes could be related to the concentration of C3 and ApoA1.

IV. CONCLUSION

The TCM has been proposed for early diagnosis of AD by measuring several types of biomarkers. Antibodies (Anti-ApoA1 and Anti-C3) were immobilized on the sensing plate using avidin-biotin conjugation to measure the ApoA1 and C3, respectively. The change in the amplitude of terahertz wave from the sensing plate depended on the concentration of C3 and ApoA1 in the 30 μl solutions with the concentration ranges of from 1 to 100 and from 0.1 to 10 μg/ml, respectively. These results suggest the TCM is one of potential tools for AD diagnosis.

REFERENCES

[1] K. Uchida et al., “Amyloid-β sequester proteins as blood-based biomarkers of cognitive decline.”, Amst., 1(2), pp. 270-280, 2015.

[2] T. Kiwa et al., “Chemical sensing plate with a laser-terahertz monitoring system”, Appl. Opt., 47(18), pp. 3324-3327, 2008.

[3] T. Kiwa et al., “Terahertz chemical microscope for label-free detection of protein complex”, Appl. Phys. Lett., 96 (21), 211114, 2010.

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