Signal Accuracy of Terahertz Chemical Microscope for Lung Cancer Cell Detection

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***Abstract*—It is essential to evaluate the number of cancer cells to analyze cancer genome for cancer genomic medicine. However, it takes a long time, and pathologists' skills are needed to evaluate it. Our group has developed and proposed a Terahertz Chemical Microscope (TCM) to evaluate it easily and fast. In this study, antibodies were immobilized by covalent bonding and avidin-biotin reaction to detect cancer cells, and their immobilizing methods were compared. The results suggest that signal accuracy is improved to detect lung cancer cells using avidin-biotin reaction.**

Keywords-terahertz chemical microscope; terahertz; immobilizing antibody; cancer cell

I. INTRODUCTION

Recently, cancer genomic medicine has been attracted to be a cancer treatment to reduce the physical burden of patients in cancer treatments. The cancer genome is analyzed, and individualized treatment is provided to each cancer patient. In order to provide cancer patients with this treatment, it is essential to analyze the cancer genome. Before analyzing the cancer genome, it is essential to evaluate the number of cancer cells in a specimen tissue to analyze the cancer genome efficiently. In general, the evaluation is done by treating the specimen tissue with Formalin-Fixed Paraffin-Embedded (FFPE) and observing it with a microscope by pathologists. However, in FFPE, the recommended time to fix the specimen tissue is from 24 to 48 hours [1], so it takes at least 2 or 3 days to evaluate. Also, evaluation precision depends on the pathologists' skills to process the tissue by FFPE or distinguish cancer cells from normal cells in the tissue.

Our group has developed and proposed a Terahertz Chemical Microscope (TCM) [2] [3] to evaluate the number of cancer cells in a solution without complex pretreatments as FFPE [4]-[6]. TCM can measure cancer cells in the solution, so little pretreatment is required. Also, TCM can evaluate the number of cancer cells quantitatively. In the TCM, cancer cells are detected using immune reactions. So, it is crucial to immobilize antibodies on a sensing plate used as a terahertz emitter.

In this study, in order to accurate detection terahertz signals, the methods of immobilizing antibodies using covalent bonding [7] [8] and using an avidin- biotin reaction [6] were compared by measuring PC9 and EBC1, which are a human lung adenocarcinoma culture cell and a human lung squamous cell carcinoma culture cell, respectively.

II. EXPERIMENTAL

TCM can detect cancer cells in a solution on a sensing plate by measuring the amplitude of terahertz wave radiated from the sensing plate. Figures 1 (a) and (b) show the processes of immobilizing antibodies using covalent bonding and avidin-biotin reaction respectively. In Figure 1 (a), 20 μg/mL of anti IgG (Antigen Affinity Purified, Bethyl Laboratories, Inc., Montgomery, Alabama, USA) was immobilized on the sensing plate using amino coupling as the covalent bonding [7] [8] for 17 h at 4 ºC, and the SiO_2 film surface was blocked using skim milk to prevent nonspecific adsorption for 15 min at 18 ºC-25 ºC. Cytokeratin, AE1/AE3 (Agilent Technologies Japan, Ltd., Tokyo, Japan) which is reacted with various tumors such as adenocarcinoma and squamous cell carcinoma was used as an antibody and it was immobilized by reacting with the anti IgG for 9 h at 4 ºC. In Figure 1 (b), 45 μg/mL of avidin (affinity-purified, Vector Laboratories, Inc., Burlingame, California, United States) was immobilized on the sensing plate for 17 h at 4 ºC, and the SiO_2 film surface was blocked in the same way as immobilizing the anti IgG. 100 μg/mL of biotin conjugated anti IgG (Antigen Affinity Purified, Bethyl Laboratories, Inc., Montgomery, Alabama, USA) was immobilized for 5 h at 4 ºC, and Cytokeratin, AE1/AE3 was immobilized in the same way as the covalent bonding. After that, in the covalent bonding and

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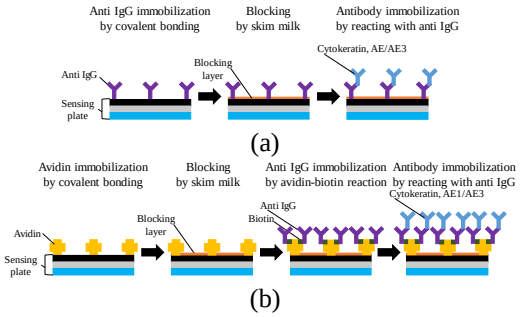

Figure 1. The processes of immobilizing antibodies on the SiO_2 film surface on the sensing plate: (a) Covalent bonding method (b) avidin-biotin reaction method

the avidin-biotin reaction, 6.7×10^6 cell/mL of PC9 and 2.7×10^6 cell/mL of EBC1 reacted with immobilized Cytokeratin, AE/AE3 for 12 h at 4 ºC, respectively. The unreacted PC9 and EBC1 were removed by washing 10 times. After Cytokeratin, AE1/AE3 was immobilized on the sensing plate and the unreacted PC9 and EBC1 were washed, the amplitude of terahertz was measured by the TCM. The change of terahertz amplitude was calculated.

III. Results and Discussion

Figure 2 shows the average change of terahertz amplitude before and after the reaction of PC9 and EBC1 in each immobilizing method. Error bars show the standard deviation of the change of terahertz amplitude in 3 samples. In the covalent bonding, the average changes of terahertz amplitude were 0.90±0.66 mV for measuring PC9 and 0.68±0.43 mV for measuring EBC1. In the avidin-biotin reaction, they were 0.82±0.15 mV, 0.33±0.23 mV for measuring PC9 and EBC1, respectively. In both methods, the average changes of terahertz amplitude in measuring EBC1 were lower than measuring PC9, because we think the concentration of EBC1 is lower than that of PC9 and the charge of EBC1 is smaller than that of PC9. Especially in the avidin-biotin reaction, we think that the lower electric charge of EBC1 was not transmitted well, because the distance between the sensing plate and the EBC1 was longer. However, the avidin-biotin reaction was 4.4-fold and 1.9-fold lower standard deviation than the covalent bonding for the measurement of PC9 and EBC1, respectively, because we think that the antibody could be

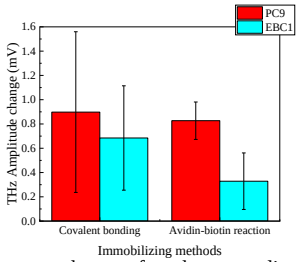

Figure 2. The average change of terahertz amplitude before and after the reaction of lung cancer cells which are PC9 and EBC1 in each immobilizing method

immobilized in higher density using avidin biotin reaction and did not detach from the sensing plate in the process of the reaction of lung cancer cells or washing. This result shows lung cancer cells could be measured accurately by immobilizing antibodies using the avidin-biotin reaction.

IV. Conclusion

Immobilization methods of covalent bonding and avidin-biotin reaction were compared by measuring PC9 and EBC1. In the avidin-biotin reaction, the change of terahertz amplitude was smaller. However, the standard deviation was 4.4-fold and 1.9-fold lower than the covalent bonding. This result suggests that lung cancer cells can be measured accurately by using avidin-biotin reaction and immobilizing the antibody in high density and firmly.

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