学 位 論 文 審 査 の 要 旨

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Development of Paper-Based Analytical Devices (μPADs) for Low-Cost, Rapid, and Ultrasensitive Enzyme-Linked Immunosorbent Assay (ELISA)
（低コスト、迅速、超高感度の酵素免疫測定法（ELISA）のための紙ベースの分析装置（μPAD）の開発）

Sandwich ELISA is a widely used analytical technique for the detection of antibodies and proteins (antigens) in biological samples. Typically, sandwich ELISA is performed using a 96-well microplate, where a capture antibody (cAb) specific to the target antigen is immobilized on the surface of the wells. When the sample is added to the wells, the antigen binds to the immobilized cAb. After an appropriate washing step, an enzyme-labeled detection antibody (dAb) is added, which binds to the antigen. This is followed by a second washing step, and then a substrate is added that reacts with the enzyme to produce a signal. The intensity of this signal corresponds to the concentration of the antigen.
Although conventional sandwich ELISA offers high specificity and sensitivity, it has several drawbacks. For instance, immobilizing the cAb typically requires overnight incubation, making the process time-consuming. In addition, large volumes of samples and reagents are needed, signal detection requires an expensive microplate reader, and the procedure must be performed by highly trained personnel. To overcome these limitations, microfluidic paper-based analytical devices (μPADs) present a highly attractive alternative. Since μPADs were introduced by the Whitesides group in 2007, they have gained significant attention from researchers as a means to simplify complex analytical techniques for various applications. By integrating ELISA with μPADs, it becomes possible to combine the high sensitivity and specificity of ELISA with the simplicity and portability of μPADs.

The summary and main achievements of this doctoral dissertation are outlined below.

The author developed a rapid, simple, and low-cost sandwich ELISA-based μPAD for the detection of the β'-component protein, a major allergenic protein found in salmon roe. This μPAD employs a convenient and rapid method for immobilizing the capture antibody, reducing the immobilization time from overnight (as in conventional ELISA) to just 5 minutes. Specifically, the capture antibody is immobilized by drying it at 50 ℃ for 5 minutes. In addition, the assay conditions were optimized to minimize the total analysis time while maintaining sufficient sensitivity. As a result, the μPAD-ELISA assay could be completed in 15 minutes, showing good linearity over a β'-component concentration range of 0.10-60.0 ng/mL, with a detection limit of 1.59 ng/mL. Compared to conventional ELISA targeting the same analyte, the developed μPAD-ELISA requires smaller volumes of reagents and significantly

shorter assay times. These advantages suggest that this method can contribute to the rapid and sensitive detection of food allergens in processed foods and the establishment of a reliable food allergy labeling system.

Subsequently, the author developed a simple, rapid, and ultra-sensitive paper-based biosensing platform integrated into a custom-designed 3D-printed microplate for the bioluminescent detection of the breast cancer biomarker HER2. This platform combines the advantages of microporous cellulose paper, which enables efficient antibody immobilization via passive adsorption within minutes without surface modification, and a reusable microplate structure capable of high-throughput analysis. The assay can be completed in just 20 minutes, a significant reduction compared to the typical 5-hour procedure required by conventional ELISA methods. The 3D-printed microplate was designed with two key features: integrated microvalves and adjustable surface wettability via surfactant treatment. These functions allow precise fluid control, enabling the retention of samples and reagents during incubation, and selective drainage of wash buffers through an absorption pad during the rinsing process. The microvalves, embedded at the bottom of each well, serve dual roles—retaining liquid during reactions and facilitating drainage during washing. The device dimensions are compatible with commercially available plate readers, allowing easy integration into existing laboratory workflows. To ensure optimal microvalve performance and device reusability, a polyethylene glycol diacrylate (PEGDA)-based resin was used for 3D printing. This material offers an ideal balance of mechanical strength, chemical resistance, and biocompatibility, supporting both effective fluid handling and repeated use. After each assay, only the paper discs need to be replaced, making the system both cost-effective and sustainable. Bioluminescence detection was performed using a sandwich immunoassay with NanoLuc luciferase as the signal reporter, which provided signal intensities approximately 100 times greater than those obtained with conventional luciferases. A strong linear correlation was observed between the logarithmic concentration of HER2 and the bioluminescence output over a wide dynamic range of 0.1-1000 pg/mL. The assay achieved an extremely low limit of detection (LOD) of 1.3 fg/mL, demonstrating 10,000-fold higher sensitivity compared to commercial colorimetric ELISA kits. Furthermore, validation using HER2-spiked human serum samples (5–800 pg/mL) showed excellent reproducibility (coefficient of variation of 9.5%) and recovery rates ranging from 86% to 108%. On the other hand, due to the small reagent volume (5 µL), the system is susceptible to pipetting errors, indicating that incorporating precision dispensing technologies could further enhance assay reproducibility. The modular platform developed in this study, combining passive capillary-driven microfluidics, the simplicity of paper-based systems, precise fluid control, and highly sensitive bioluminescent detection, offers a powerful and low-cost solution for rapid, quantitative biomarker detection. This system holds strong promise for applications in the early diagnosis of cancers, infectious diseases, and other clinically significant conditions.

In summary, the author developed a high-performance paper-based microfluidic device technology to realize sandwich ELISA. This achievement successfully overcomes the limitations of conventional ELISA through microfluidic device technology. It holds great promise not only for applications in analytical chemistry, medical, and biological research but also for practical implementation. Therefore, the author is deemed qualified to be awarded the degree of Doctor of Philosophy in Chemical Sciences and Engineering from Hokkaido University.