学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

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 common analytical technique used for the detection of antibodies and proteins or antigens in biological samples. Typically, sandwich ELISA is performed using a 96-microwell plate, where a capture antibody (cAb) specific for the required antigen is immobilized on the surface of the wells. When the sample is added to the wells, the antigen attached to the immobilized cAb. Then, after a suitable washing process, another enzyme-labeled detection antibody (dAb) is added to attach to the antigen. This is followed by a second washing, and a substrate is added, which reacts with the enzyme and produces a signal that is related to the antigen concentration. Conventional sandwich ELISA is a specific and sensitive technique; however, it is time-consuming because overnight incubation is needed to immobilize cAb, and it has other disadvantages that large sample and reagent volumes are required, the microwell plate reader used for signal readout is expensive, and a highly trained analyst must carry out the work. Microfluidic paper-based analytical devices (μPADs) are a very attractive approach for overcoming all these disadvantages. Since the introduction of μPADs by Whitesides' group in 2007, μPADs have attracted huge interest from researchers as ways to simplify complex analytical techniques in different applications. by the integration between ELISA and μPADs, we can have the high sensitivity and specificity of the ELISA assays with the simplicity and portability of μPADs. In Chapter General Introduction, I discussed the current perspectives of μPADs, their fabrication, applications, and challenges. Also, the current perspective of ELISA-based μPADs was discussed.

In Chapter 2, a rapid, simple, and low-cost sandwich ELISA-based μPAD is presented for the determination of β'-component protein, a major allergenic protein in salmon roe. This μPAD employed a simple and rapid method to immobilize the capture antibody which reduced the immobilization time to 5 minutes instead of overnight for conventional ELISA. The immobilization was done by drying the capture antibody at 50 ℃ for just 5 min. The assay parameters were optimized to achieve the optimal sensitivity with a minimal assay time. Under the optimal conditions, the μPAD-ELISA assay took 15 minutes and it provided consistent performance over a wide range of β'-component concentrations: there was a good linear relationship over a range of 0.10–60.0 ng mL-1 and a limit of detection of 1.59 ng mL-1. Compared to the conventional ELISA assay for the same target, the developed μPAD-ELISA requires minimal reagent volumes and shorter assay times. These advantages are expected to contribute to the rapid and sensitive detection of food allergens in processed food as well as the establishment of a good food allergy labeling system.

In Chapter 3, I introduced a simple, rapid, and ultra-sensitive paper-based biosensing platform integrated into a custom-designed 3D-printed microplate for the bioluminescence detection of the breast cancer biomarker HER2. This hybrid system combines the advantages of microporous cellulose paper, which enables efficient antibody immobilization via passive adsorption within minutes and without requiring surface modification, and a reusable microplate architecture that supports high-throughput analysis. The assay is completed in just 20 minutes—significantly shorter than the typical 5-hour duration of conventional ELISA

methods. The 3D-printed microplate was engineered with two critical features: embedded microvalves and modifiable surface wettability via surfactant treatment. These functionalities allow precise fluid control—enabling retention of sample and reagent solutions during incubation while facilitating selective drainage of washing buffer through an adsorption pad during rinsing. The microvalves are integrated at the base of each well and play a dual role in retaining fluids during reaction steps and allowing buffer flow during washing. The device dimensions are compatible with standard commercial plate readers, allowing easy integration into existing laboratory workflows. To ensure optimal valve performance and device reusability, a polyethylene glycol diacrylate (PEGDA)-based resin was selected for 3D printing. This material provides an ideal combination of mechanical strength, chemical resistance, and biocompatibility, supporting both fluid handling and repeated use. After each assay, only the paper discs are replaced, making the platform cost-effective and sustainable. Bioluminescence detection is carried out using a sandwich immunoassay with NanoLuc luciferase as the signal reporter, delivering approximately 100-fold greater signal intensity compared to traditional luciferases. A strong linear correlation was observed between the bioluminescence output and the logarithmic concentration of HER2 over a wide dynamic range (0.1–1000 pg/mL). The assay achieved a remarkably low limit of detection (LOD) of 1.3 fg/mL—representing a 10,000-fold improvement over commercial colorimetric ELISA kits and traditional platforms. Validation using HER2-spiked human serum (5–800 pg/mL) showed excellent reproducibility (CV ~ 9.5%) and recovery rates between 86% and 108%. While the small reagent volume used (5 µL) contributes to the assay's sensitivity, it also makes the system highly susceptible to pipetting errors, suggesting that precision dispensing could further improve the assay's reproducibility. This modular paper-in-3D-printed microplate platform—combining passive capillary-driven microfluidics, paper-based simplicity, precise fluidic control, and high-sensitivity bioluminescent readout—offers a powerful and low-cost solution for rapid, quantitative biomarker detection. The system demonstrates strong potential for applications in the early diagnosis of cancers, infectious diseases, and other clinically relevant conditions.

In the last chapter, a summary of the results and findings from the research was discussed. Moreover, the challenges and the future prospects were introduced.