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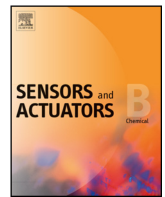

A novel insight in rapid allergen detection in food systems: From threshold dose to real-world concentration

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ABSTRACT

Recently, food safety application of microfluidic device has drawn increasing attentions, whereas the research in this field is still limited due to the complexity of food systems. Most of immunosensor technology has endeavored to improve the sensitivity of the assay. However, the major challenges in food allergen detection are to simplify detection by creating a device with both rapid and broaden detection range from threshold to the real-world concentration. In this work, an ELISA-based colorimetric detection of allergen in food systems was achieved using microfluidic device and regular optical microscope. Casein, the major allergen in milk, was selected as a model analyte. The results indicated that the device was superior to the traditional method and substantially simplified the allergen detection, by demonstrating rapid detection, wide detection range from threshold to real-world concentration. The total detection time was reduced substantially from 45 min to 15 min without diminishing the detection limit. The linear detection range of antigen was also increased by 5-log difference. Moreover, this study also investigated the mechanism and kinetic study of TMB in-channel oxidation, and established a protocol to determine microfluidic channel size and TMB incubation time for allergen detection in food systems. Therefore, this work not only demonstrated the capability and superiority of microfluidic device for food safety application, but also provided a general optimization method for rapid allergen detection in food systems.

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1. Introduction

Food allergy has become one of the major health and food safety issues all over the world. Food allergy is an adverse immunological response due to allergic substances in food, which occurs in about 1–3% in the adult and 4–6% in children population [1]. In U.S., for instance, approximately 30,000 patients are hospitalized each year due to food allergy irritation, which also causes 150–200 lethal cases [1]. According to the FDA definition, food allergy is different from food intolerance or other non-allergic reactions. Food intolerance is an abnormal response, without the involvement of human immune system, to a food component or additive, such as gluten-sensitive enteropathy [1,2]. However, food allergen can provoke the massive production of allergen specified IgE, IgA antibody, or T cells, which specifically react to allergic substances by the renowned antibody–antigen (ab–ag) reaction. Because of the high sensitivity of these types of ab–ag reaction, even the intake of trace amounts of food allergens can cause severe allergic symptoms, including digestive disorder, respiratory symptoms, circulatory symptoms, and skin irritations. For some sensitized individuals, contact with specific food allergens can even lead to life-threatening situations (anaphylactic shock). Therefore, in recent years, a series of legislative amendments required food labels to include clear statement about whether the food products contain a major food allergen, e.g. milk, eggs, peanuts, tree nuts, soybeans, wheat, fish and shellfish, which account for 90% of food allergies [3]. Besides, it is also necessary to guarantee the presence of allergens and precisely determine the trace amount of allergens in food products, which is not only beneficial to the allergic consumers, but also in the economic concern of food manufacturers. To fulfill this objective, reliable analytical tools should be developed to ensure simply detection and time/cost efficiency.

Most methods developed involve ab–ag reaction using bioactive proteins, which can specifically react to either allergen itself or a biomarker indicating the presence of allergic substances. Due to the simplicity of measurement and cost-effectiveness of the instruments involved, Enzyme-linked immunosorbent assay (ELISA) is the most widely accepted method and many commercially available food allergen detection kits are based on this method. Although the threshold dose for food allergens varies tremendously among

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Table 1
Commercial allergen detection kits for milk [2].

Name	Target	Test format	Detection limit	Duration
RAPID 3-D Casein test kit (Tepnel)	Casein	LFA	N/A	10 min
Reveal for Total Milk Allergen (Neogen)	Casein and whey	LFA	5 μg/mL	5 min
Casein Residue ELISA	Casein	ELISA	1	45 min
RIDASCREEN FAST Casein (R-Biopharm)	Casein	ELISA	500 ng/mL	30 min
Beta Lactoglobulin Residue ELISA	β-Lacto globulin	ELISA	0.1	45 min
Alert for Total Milk Allergen (Neogen)	Milk protein	ELISA	5 μg/mL	30 min
Veratox for Total Milk Allergen (Neogen)	Milk protein	ELISA	5 μg/mL	30 min

individual allergens, a general agreement to the commercial kits sets the detection limit to 'parts per million' (ppm). In common commodities, allergen content ranges up to 1% in liquid sample and 30% in solid product. A list of commercial kits for allergen detection is summarized in Table 1. The methods are developed either in 96-well plate ELISA or lateral-flow immune-chromatographic assay (LFA) assay involving protein-based ab–ag reaction. The LFA method is cost-effective that do not require additional analytical instrument and has high-sensitivity and time-efficiency, whereas it can only perform qualitative analysis. Contrarily, the commercial ELISA kits are quantitative methods with high sensitivity based on ultraviolet visible (UV–vis) or fluorescent spectrophotometer, but the major limitation is their low time-efficiency and narrow detection range (4). Therefore, since both LFA and 96-well based ELISA have crucial limitations, this study will focus on developing a novel platform, based on microfluidics, for quantitative, high-sensitive and time/cost effective food allergen detection.

Microfluidics is an emerging technology for chemical/biological synthesis or molecular analysis [4,5]. Two advantages of microfluidic-based assay are that it can perform traditional laboratory operation using substantially less amount of reagents (from mL to μL) and less amount of time (from hours to seconds) [4]. In recent years, microfluidic chips have been proven to have the capability to perform a variety of analytical measurements, including ELISA [6]. In several recent review articles [7–9], researchers have proposed a promising future for the application of microfluidic device for food safety application. However, the real application of microfluidics for food allergen is still limited. The application of microfluidics device for food safety is different from that in biomedical field, which was primarily targeted for improved sensitivity for early diagnosis. The major challenges for food allergen detection were attributed to the long detection range, high allergen content, and the complexity of food matrix [7]. In this study, the major allergen in bovine milk, casein, was selected as a model allergen. The threshold dose for casein allergen, determined as lowest observed adverse-effect level (LOAEL), is generally considered at 1–10 ppm. In common commodities containing dairy components, the casein content ranges up to 25 mg/g in liquid product and 30 wt% in solid product. In current study, we have developed an ELISA-based colorimetric method to illustrate how both wide detection range and short detection time were achieved on a microfluidic biosensor for casein.

2. Materials and methods

2.1. Reagents and instruments

The SU-8 2050 and 2075 photoresists were purchased from MicroChem (Newton, MA, USA). The polydimethylsiloxane (PDMS) elastomer kit (Sylgard 184) was purchased from Dow Chem. (Midland, MI, USA). Sheep polyclonal anti-casein antibody (primary antibody), and chromogenic ultra 3,3,5,5-tetramethylbenzidine (TMB) substrate were purchased from Pierce Antibodies (Thermal Scientific, Rockford, IL). Rabbit anti-casein polyclonal antibody with horseradish peroxides (HRP) label (secondary antibody) was purchased from Bioss Inc. (Woburn, MA, USA). The antibodies and TMB substrates were used in both microfluidic and 96-well plate methods. All other chemicals and Si wafers were obtained from Sigma Aldrich (St. Louis, MO, USA). The fabricated channels were examined by a profilometer (AlphaStep 200, KLA-Tencor, Milpitas, CA, USA). The on-channel colorimetric data were collected from an optical microscope (MVX10, OLYMPUS, JAPAN), while the absorbance of 96-well plate was measured on a multilabel plate reader (VICTOR X3, Perkin Elmer, Waltham, MA, USA). A peristaltic pump (M2-2/12, Ismatec Reglo Digital, Germany) was used for sample delivery and rinsing.

2.2. Microfabrication and functionalization of PDMS device

High-resolution transparency mask was first printed according to a double T-junction layout design (Fig. 1a). The Si master wafer was created by contact photolithography with SU-8 2050 photoresist on a mask aligner according to the supplier's instruction. PDMS replicas were produced by curing PDMS mixture (base: curing agents = 10:1) on Si master wafer at 125 °C for 20 min. Cured PDMS replicas were then bound to clean glass slides via O_2 plasma treatment (March Jupiter III, Westlake, OH, USA). The PDMS/glass microfluidics devices with metal needle ports were cured and bound following previous protocol [10]. PDMS microchannels were then pre-treated and functionalized with grafted dextran layer by pumping through 3-aminopropyltriethoxylsilane (APTES), dextran, and sodium periodate ($NaIO_4$) solutions to form covalent imine bonds(C=N), following Yu's method [11].

2.3. On-chip ELISA assay

The antibodies were then immobilized in the pre-treated PDMS channels through covalent imine bonds between the amine on antibody and the aldehyde on grafted dextran. First, a 10 μg/mL anti-casein PBS solution was pumped into the device to allow the binding of primary antibody. The incubation lasted for 5 min at the flow rate of 28 μL/min, which was followed by PBS rinsing for 1 min to remove unbounded antibody. Then, the channels were treated by 1 mg/mL of BSA in PBS buffer for 5 min to block any unreacted dextran surface. After rinsing with PBS for 1 min, casein standard solution at concentration of 100, 200, 500 ng/mL, 1, 2, 5, 10, 20, 50, 100, 200, 500 μg/mL, and 1 mg/mL were then individually pumped at a flow rate of 28 μL/min for 5 min to allow the specific ab–ag reaction between casein analyte and immobilized primary anti-casein antibody. After rinsing with PBS for 1 min, the ab–ag conjugates were quantified by secondary antibody, which was HRP-labeled polyclonal anti-casein antibody (10 μg/mL). The incubation of secondary antibody was conducted at 28 μL/min for 5 min, followed by 1 min of PBS rinsing. Finally, TMB substrate was pumped into the PDMS channels at a rate of 28 μL/min. The color change was captured over time by optical microscope (OLYMPUS MVX10). Colorimetric analysis of PDMS channel was conducted by

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