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Point-of-use ultrafast single-step detection of food contaminants: a novel microfluidic fluorescence-based immunoassay with integrated photodetection

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Abstract

The assurance of strict food safety standards is a major challenge in modern society. Mycotoxins, in particular, are produced by fungi during production, storage or transport, posing serious health concerns to humans and animals. Aiming at developing a point-of-use tool for the rapid detection of mycotoxins at the required regulatory levels, we report a microfluidic ultrafast and single-step fluorescence-based immunoassay, using minimal amounts of reagents. This assay was integrated with 200×200 µm thin-film a-Si:H photodiodes towards a compact detection system. Aflatoxin B1 was detected at 1 ng/mL after 2 min assay time, among the fastest reported in the literature.

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Keywords: ultrafast, mycotoxin, beads, fluorescence, photodiodes, real-time, microfluidics

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1. Introduction and motivation

The production and trade of food and feed products across the globe is a large and expanding market, which makes the assurance of strict food safety standards one of the major challenges in modern society. Mycotoxins, in particular, such as ochratoxin A (OTA), aflatoxin B1 (AFB1) and deoxynivalenol (DON) are produced by fungi in spot-contaminations during production, storage or transport, posing serious health concerns both to humans and animals, ranging from carcinogenicity and hepatotoxicity to decrease in weight gain. Fort this reason, mycotoxins have been subject of increasingly strict regulations, particularly in the European Union.

The monitoring of mycotoxin levels in food and feed is typically performed by sending a sample to a certified laboratory for quantification using analytical techniques such as HPLC-MS or ELISAs. These methods, while sensitive and accurate, are also lengthy (hours to days), complex and need to be performed in a laboratory by specialized personnel. In order to cope with the demand for rapid and simple point-of-use screening of mycotoxins during transport, trade and storage of food supplies, several strategies are under research. Many of these strategies rely on very complex surface chemistries and detection schemes [1], multiple sequential steps and/or need expensive equipment for quantitative analysis [2] and are often incompatible with complex food or feed matrices [3]. Therefore, in order to improve beyond our previous achievements [4] and the current state of the art, a novel ultrafast and single-step fluorescence-based competitive immunoassay, utilizing nanoporous agarose beads functionalized with protein A or G confined in a microfluidic chamber, is reported.

2. Methods

2.1. Device and immunoassay design

The working principle of the single-step fluorescence immunoassay is schematized in Figure 1-a) for the detection of AFB1. The assay principle involves a competition between (1) the toxin molecules that may be present in the sample-under-analysis (SUA) and (2) spiked AFB1-BSA-Alexa 430 conjugates for the occupation of the binding sites of an anti-AFB1 antibody, immobilized on the beads via protein A binding to the Fc region. Therefore, the higher the contamination degree on the SUA, the lower the fluorescence emission increase measured over time.

The PDMS microfluidic device is comprised of a region with a height of 100 µm where commercial protein A agarose beads are packed against a second region with a height of 20 µm. This difference in heights is responsible for physically trapping the beads when flowed through the taller channel (Figure 1-b) [5]. The chamber is aligned with a focused 405 nm laser beam for fluorophore excitation. The AFB1-BSA-Alexa 430 conjugates captured on the beads are continuously monitored by measuring the current through a 200 × 200 µm a-Si:H p-i-n photodiode at 0V bias (Figure 1-c) [6], aligned beneath the chamber, which is proportional to the emitted light with a maximum at λ=540 nm. In order to minimize interference from the excitation light, an a-SiC:H absorption filter is deposited on top of the photodiode, which strongly absorbs light in the blue-violet range with a transmittance below 0.1%, while being transparent in the green-yellow range.

2.2. Device operation and optical measurements

The online fluorescence measurements during the flow of SUA through the chamber, according to Figure 1-b), were performed using either a standard fluorescence microscope or the integrated photodiode. The fluorescence microscope measurements were performed in a Leica (Wetzlar, Germany) DMLM microscope equipped with a DFC300FX camera in 20s intervals with a light exposure time of 1s to a 100 W mercury arc lamp coupled to a blue light excitation filter with band pass between 450 and 490 nm and an emission long pass of 515 nm. The photodiode measurements were performed using a picoammeter (Keithley 237) at 0V bias voltage while continuously illuminating the chamber using the 405 nm laser.