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Lab-on-a-chip based biosensor for the real-time detection of aflatoxin

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ABSTRACT

Polymers were synthesized and utilized for aflatoxin detection coupled with a novel lab-on-a-chip biosensor: MiSens and high performance liquid chromatography (HPLC). Non-imprinted polymers (NIPs) were preferred to be designed and used due to the toxic nature of aflatoxin template and also to avoid difficult clean-up protocols. Towards an innovative miniaturized automated system, a novel biochip has been designed that consists of 6 working electrodes (1 mm diameter) with shared reference and counter electrodes. The aflatoxin detection has been achieved by a competition immunoassay that has been performed using the new biochips and the automated MiSens electrochemical biosensor device. For the assay, aflatoxin antibody has been captured on the Protein A immobilized electrode. Subsequently the sample and the enzyme-aflatoxin conjugate mixture has been injected to the electrode surfaces. The final injection of the enzyme substrate results in an amperometric signal. The sensor assays for aflatoxin B1 (AFB1) in different matrices were also performed using enzyme link immunosorbent assay (ELISA) and HPLC for confirmation. High recovery was successfully achieved in spiked wheat samples using NIP coupled HPLC and NIP coupled MiSens biosensor [2 ppb of aflatoxin was determined as 1.86 ppb (93% recovery), 1.73 ppb (86.5% recovery), 1.96 ppb (98% recovery) and 1.88 ppb (94.0% recovery) for immunoaffinity column (IAC)-HPLC, NIP-HPLC, Supel™ Tox SPE Cartridges (SUP)-HPLC and NIP-MiSens, respectively]. Aflatoxin detection in fig samples were also investigated with MiSens biosensor and the results were compared with HPLC method. The new biosensor allows real-time and on-site detection of AFB1 in foods with a rapid, sensitive, fully automated and miniaturized system and expected to have an immense economic impact for food industry.

1. Introduction

Mycotoxins are secondary metabolites mainly produced by filamentous fungi on various food and feedstuffs. Among mycotoxins, aflatoxins (AFs) are considered to be the most toxigenic fungal metabolites with carcinogenic, mutagenic, teratogenic and immunosuppressive effects. Aflatoxin is one of the most common mycotoxins in grains. The occurrence of AFs is influenced by certain environmental factors; hence, the extent of contamination varies with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during preharvest, storage, and/or processing periods [1,2]. Four types of AFs including B1, B2, G1, and G2 commonly occur in the natural environment even though 20 different AFs have been identified [3]. Aflatoxin B1 was classified as Group 1 carcinogen (carcinogenic to humans) by the International Agency for Research on Cancer (IARC) [4]. Aflatoxins have been found in grains and herb medicines in humid conditions [2]. European Commission has established maximum limits for AFs, due to their frequent presence in food and foodstuff and to minimize their toxic and health hazards effect in animals and humans. The permissible limits for AFB1 and total AFs are permitted 2 $\mu g\,kg^{-1}$ and 4 $\mu g\,kg^{-1}$, respectively in all cereals and products derived from cereals, including processed cereal products [5,6]. With the increase of regulations for acceptable levels of AFs in place, modern analytical methods have become highly sophisticated with high

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precision and accuracy, suitable for regulatory laboratories and for post-harvest sample testing in developed countries.

Solid-phase extraction (SPE) has appeared as a simple alternative to be coupled with a variety of methods for aflatoxin detection to provide easy and cost effective techniques [7]. The applications of SPE can be widely seen also in imprinting technology as it offers simple and successful clean up procedures. Molecularly imprinted polymers (MIPs) have increasingly been introduced in many analytical procedures, mainly due to their high selectivity in comparison to other stationary phases. MIPs have attracted significant attention as substitutes for natural receptors in chromatography, sensors and assays [8,9]. The reason for this is coming from the fact that molecular imprinting is arguably the most generic and cost effective technique for preparing synthetic receptors. Imprinted polymers have several important advantages as compared to natural receptor molecules: (i) polymers can be prepared for practically any compound; (ii) they have similar affinity to natural biomolecules but often have better specificity; (iii) polymers can work in organic solvents and they are stable at low/high pHs, pressure and temperature [7].

Most of the current methods for quantitative AFs determination include chromatographic methods such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and recently liquid chromatography tandem mass spectrometry (LC-MS/MS), suitable for use in regulatory laboratories [10]. Several immunology based semi-quantitative and qualitative methods including enzyme linked immunosorbent assays (ELISAs) were also developed. The novel AFs detection systems for rapid screening and detection in field and laboratory applications are dip-stick kits [11], optical-based sensing methods (e.g. hyperspectral imaging and electronic noses) [12], and biosensors [13]. Detection for regulatory purposes requires the development of validated official analytical methods. High-performance liquid chromatography (HPLC) [14], thin-layer chromatography (TLC) [15], surface plasmon resonance (SPR) and enzyme-linked immunosorbent assay (ELISA) [16] are some of the current methods for the confirmatory and quantitative determination of AFs in contaminated samples. However, these techniques require qualified personnel, time consuming sample pre-treatment and assay procedure and expensive laboratory equipments. These tests also

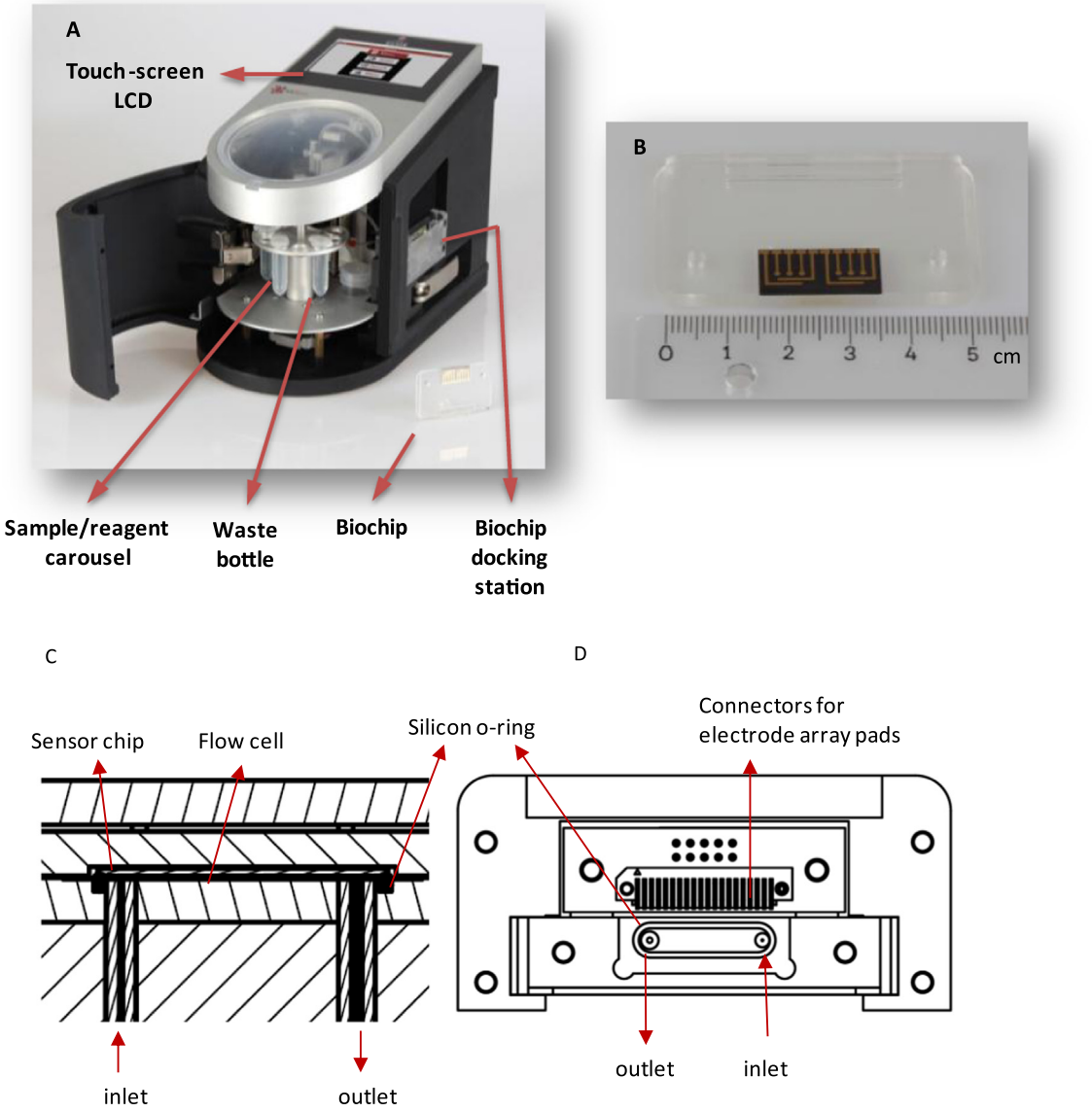

Fig. 1. A novel biosensor (A) and its sensor chip (B) designed and manufactured in-house for rapid and reliable detection of aflatoxin. The placement of the biochip to the docking station creates a microfluidic channel on electrodes and provides the electrode connection to the device (C-D).

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