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Novel glucometer-based immunosensing strategy suitable for complex systems with signal amplification using surfactant-responsive cargo release from glucose-encapsulated liposome nanocarriers

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ABSTRACT

Methods based on surfactant-responsive controlled release systems of cargoes from nanocontainers have been developed for bioanalytical applications, but most were utilized for drug delivery and a few reports were focused on immunoassays. Herein we design an *in situ* amplified immunoassay protocol for high-efficient detection of aflatoxins (aflatoxin B_1, AFB_1 used in this case) based on surfactant-responsive cargo release from glucose-encapsulated liposome nanocarriers with sensitivity enhancement. Initially, biotinylated liposome nanocarrier encapsulated with glucose was synthesized using a reverse-phase evaporation method. Thereafter, the nanocarrier was utilized as the signal-generation tag on capture antibody-coating microplate through classical biotin-avidin linkage after reaction with biotinylated detection antibody. Upon addition of buffered surfactant (1X PBS-Tween 20 buffer) into the medium, the surfactant immediately hydrolyzed the conjugated liposome, and released the encapsulated glucose from the nanocarriers, which could be quantitatively determined by using a low-cost personal glucometer (PGM). The detectable signal increased with the increment of target analyte. Under the optimal conditions, the assay could allow PGM detection toward target AFB_1 as low as 0.6 pg mL^{-1} (0.6 ppt). Moreover, the methodology also showed good reproducibility and high specificity toward target AFB_1 against other mycotoxins and proteins, and was applicable for quantitatively monitoring target AFB_1 in the complex systems, *e.g.*, naturally contaminated/spiked peanut samples and serum specimens, with the acceptable results. Taking these advantages of simplification, low cost, universality and sensitivity, our design provides a new horizon for development of advanced immunoassays in future point-of-care testing.

1. Introduction

Ongoing efforts have been made worldwide to explore innovative detection methods with the aim of manufacturing portable and affordable devices, particularly immunoassay development, while preserving the essential benefits in sensitivity, robustness and broad applicability (Creminon and Taran, 2015; Justino et al., 2015). Despite some advances for specialized point-of-care assays of interest, quantitation is usually performed in a centralized laboratory by technicians (Eberi et al., 2015; Rosenberg and Restifo, 2015; Shan et al., 2015). Hence, the whole process is time-consuming and expensive (which makes decision-making more cumbersome) and is unsuitable for routine usage. To keep pace with expectations with future point-of-care testing, there is the request to exploit more flexible, low cost, yet highly sensitive, quantitative, and easy-to-use schemes for immunoassay development.

Although the point-of-care testing devices are welcome in biological studies including clinical diagnostic, environmental monitoring and food safety, the existence of surrounding infrastructure places fewer constrains on the methodology (Kaushik et al., 2016; Zhu et al., 2015; Li et al., 2015a). Without a doubt enzyme-linked immunosorbent assay (ELISA) formats are used extensively for this purpose (Berg et al., 2015; Tang et al., 2010). Unfavorably, ELISA-based signal readouts are usually executed with expensive instrumentations by skilled operators, *e.g.*, UV–vis adsorption spectroscopy, surface plasmon resonance (SPR), quartz crystal microbalance (QCM), fluorescence, chemiluminescence and electrochemical method (Akhavan-Tafti et al., 2013; Hu et al.,

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2012; Lai et al., 2014; Tang and Ren, 2008; Algaar et al., 2015). Despite the simplicity and high-speed of the immunoprecipitation, colorimetric detection and immunochromatography assay (*e.g.*, lateral-flow immunodipstick), they only acquire a qualitative or semi-quantitative detection result (Lai et al., 2015; Yetisen et al., 2013; Gauglitz, 2014). In contrast, the rapidly emerging research field of personal glucometer (PGM, one of the most widely used diagnostic devices) opens a new horizon for the development of point-of-care testing because of its portable size, easy operation, low cost and reliable quantitative results (Zhao et al., 2015; Du et al., 2015a). The Lu group reported a series of glucometer-based detection methods for screening of metal ions, small molecules and proteins (Gu et al., 2015; Tian et al., 2015; Zhou et al., 2014; Xiang and Lu, 2014; Xiang and Lu, 2011; 2012a; 2012b; 2013). Unfortunately, introduction of natural enzymes (*e.g.*, invertase and amylase) was indispensable in the detection system since enzymatic catalytic efficiency is susceptible to interference and assay conditions at the signal-generation stage (*e.g.,* pH, temperature and instability cause by structural unfolding) (Wild, 2005). Moreover, the signal readout for enzyme immunoassays must be controlled and preserved free from interferences in much the same as the antigen-antibody reaction (Zhang et al., 2013). To this end, our groups recently designed two glucometer-based homogeneous immunosensing strategies for the detection of toxins based on target-responsive cargo release from glucose-loaded mesoporous nanocontainers with a one-step competitive-type displacement reaction mode (Tang et al., 2014; Gao et al., 2014). Nevertheless, one of the biggest flaws on the strategies could not be applied for the determination of the samples containing endogenous glucose since it might interfere with the final assay results without the sample separation and washing steps. In this regard, our motivation in this work is to construct glucometer-based immunosensing protocols suitable for quantitative detection of target analyte in the complex systems.

Another important concern for immunoassay development is to achieve low limits of detection and quantification. Recent research has looked to develop innovative and powerful nanostructures by controlling and tailoring their properties in a very predictable manner to meet the requirement of specific applications (Pei et al., 2013; Tang et al., 2013; Ranzoni et al., 2015). Liposome, a spherical vesicle having at least one lipid bilayer, can be used as a nanovehicle to encapsulate the biomolecules or indicators (*e.g.*, enzyme, dye, quantum dots, gold nanoparticles and fluorescence molecules) in its lumen because of the capacity in the aqueous core together with the phospholipid head groups (Li et al., 2015b; Cui et al., 2015; Zhou and Li, 2015; Du et al., 2015b; Kim et al., 2015). Significantly, the indicator-encapsulated liposomes are easily hydrolyzed through the added hydrolytic agents or surfactants, thereby causing the release of numerous indicators from the liposome nanovehicles (Ngo et al., 2008; Lichtenberg and Barenholz, 1998). The released indicators can be monitored by using different signal transducers, *e.g.*, ion-selective electrode, amperometery and spectroscopy (Tabaei et al., 2013; Kim et al., 2008; Katsu, 1993; Caracciolo, 2015; Franzen and Qstergaard, 2012). Inspired by these advantages, we herein use aflatoxin B_1 (AFB_1, one of toxic secondary metabolites produced by various mold species and contaminated in many agricultural commodities) as a model analyte, and design a simple, low-cost and portable glucometer-based immunosensing platform for quantitative monitoring of AFB_1 in the complex systems with the signal amplification by using surfactant-responsive release of cargo from glucose-encapsulated liposome nanocarriers. Initially, the sandwiched immunocomplex is formed with the biotinylated detection antibody on anti-AFB_1 capture antibody-coated microplate in the presence of target AFB_1. Then, the biotinylated liposome containing glucose molecules is immobilized onto the plate *via* the classical biotin-avidin linkage. Upon introduction of 1X PBS-Tween 20 into the microplate, the surfactant induces immediately the liposome hydrolysis, leading to the release of the encapsulated glucose from the nanoliposomes, which can be quantitatively determined *via* a personal glucometer. Introduction of subsequent glucose-encapsulated liposome after the antigen-antibody reaction is favorable for the detection of target analyte in the presence of endogenous glucose. The aim of this study is to set up a universal and user-friendly point-of-care testing device for the development of advanced immunoassays in clinic diagnostics, food safety analysis and environmental monitoring.

2. Experimental

2.1. Material and reagent

Polyclonal anti-AFB_1 antibody produced in rabbit (Product no.: A8679, fractionated antiserum, buffered aqueous solution. Polyclonal anti-AFB_1 antibody was not only used as the capture antibody, but as the detection antibody for design of the sandwiched immunoassay according to the guideline of this product: https://www.sigmaaldrich.com/catalog/product/sigma/a8679?lang=zh&region=CN) (Immunogen: aflatoxin B_1-KLH; Physical form: 0.01 M pH 7.4 phosphate-buffered saline solution containing 15 mM sodium azide) and AFB_1 standards from *Aspergillus flavus* (product no.: A6636) were purchased from Sigma-Aldrich. Biotin ($\geq$99%, lyophilized powder), streptavidin from *Streptomyces avidinii* (affinity purified, lyophilized from 10 mM potassium phosphate), *N*-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethyleaminopropyl)carbodiimide hydrochloride (EDC) and bovine serum albumin (BSA) were also achieved from Sigma-Aldrich. Hydrogenated Soybean Phospholipids, cholesterol and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*- biotinyl[poly(ethylene glycol)]-2000 were acquired from Avanti Polar Lipids Inc. (Alabama, USA). All high-binding polystyrene 96-well microplates were achieved from Greiner Bio-One (705071, Frickenhausen, Germany). Pierce™ 20X PBS Tween 20 buffer (Thermo Sci. Inc., USA) was diluted 20-fold with pure water to yield 10 mM sodium phosphate containing 0.15 M NaCl and 0.05% Tween-20, pH 7.4, which was proceeded with this experiment (designated as PBSTw-1X). All other reagents were of analytical grade. Ultrapure water from a Millipore water purification system (18.2 MΩ cm^{-1}, Milli-Q, Merck Millipore, Darmstadt, Germany) was used in all runs. Phosphate buffer solution (PBS buffer, pH 7.4) contained 10 mM phosphate-buffered saline, 0.137 M NaCl and 0.03 M KCl. The binding/washing buffer consisted of PBS solution with the added 0.05% (w/v) Tween 20. The blocking buffer was 2.5 wt% BSA in PBS buffer. Personal glucometer (PGM) was purchased from Roche (Accu-Chem® Active, Selangor Darul Ehsan, Malaysia).

2.2. Preparation of biotin-conjugated anti-AFB_1 antibody

Biotin-conjugated anti-AFB_1 antibody (*i.e.*, biotinylated detection antibody) was synthesized through the carbodiimide coupling similar to our previous reports (Tang et al., 2009; 2012). Initially, 700 μL of 50 mM HEPES buffer (pH 9.3) containing 2.76-mg biotin ($\geq$99 wt%) was adjusted to pH 7.2–7.4 by direct using 3.0 M HCl. Then, 11-mg NHS and 15-mg EDC were dissolved in the mixture followed by continuous shaking on an end-to-end shaker for 60 min at room temperature (RT). After that, 300 μL of anti-AFB_1 antibody in 50 mM pH 7.4 HEPES (0.1 mg mL^{-1}) was slowly dropped to the resulting mixture under gentle stirring and left for 12 h at RT (*Note*: The amount of biotin should be excess relative to the antibody). The as-produced conjugates were centrifuged for 10 min at 5000 *g* to remove the precipitates. Finally, the obtained