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A photonic crystal biosensor assay for ferritin utilizing iron-oxide nanoparticles

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ABSTRACT

Iron deficiency anemia afflicts 1 in 3 individuals, mostly women and children worldwide. A novel application using iron-oxide nanoparticles (IONPs) and a photonic crystal (PC) optical biosensor as an immunodiagnostic platform for detection of serum ferritin, a biomarker for iron deficiency, is presented. Human liver ferritin (450 kDa), clinical serum controls, and three commercially available ferritin ELISA tests were used to evaluate the PC biosensor assay in terms of inter- and intra-assay variability, spike-recovery (%), limit of detection (LOD), and matrix effects on binding. For the PC biosensor, signal response from label-free, sandwich with secondary antibody (pAb), and pAb functionalized with iron-oxide nanoparticles (FpAb) assays were detected using the Biomolecular Interaction Detection (BIND) system. Bland–Altman analysis was used to evaluate agreement between expected values for ferritin in control sera and each of the detection platforms. Inter- and intra-assay variability of the PC biosensor were both $<10\%$. Percent mean recovery ($\pm$%RSD) of ferritin from two control sera samples were 94.3% (13.1%) and 96.9% (7.6%). Use of FpAb in PC biosensor resulted in two orders of magnitude increase in sensitivity compared to label-free assay; capable of measuring serum ferritin as low as 26 ng/mL. In comparison to ELISA tests, the PC biosensor assay had the lowest bias (-1.26; 95% CI [-3.0–5.5]) and narrower limit of agreement (-11.6–9.1 ng/mL) when determining ferritin concentrations from control sera. These proof-of-concept studies support the use of IONPs to enhance detection sensitivity of PC biosensors for determination of biomarkers of nutritional status.

1. Introduction

Iron-deficiency anemia is the most prevalent micronutrient deficiency afflicting 1 in 3 people worldwide; most of them women and children who live in rural areas of developing countries (Black et al., 2008). Iron-deficiency anemia causes deleterious effects on pregnancy outcomes, children's cognitive and physical development, and productivity in working adults (Black et al., 2008). The identification of populations suffering from iron deficiency, however, remains a significant limitation that hinders nutrition and health improvements.

Despite their widespread use and availability, commercial test platforms like enzyme linked immunosorbent assays (ELISA) and radioimmunoassays (RIA) are expensive and impractical in field settings considering the high cost of equipment (plate reader, glassware), the need for specialized resources in the field (electricity, radioactive reagents), and the need for highly trained personnel (nurses and technicians) (Nash et al., 2012). On the contrary, robust and field-friendly technologies in biosensing demonstrate potential for point-of-care nutrition diagnostic methodologies.

Photonic crystals are periodic dielectric surface structures, designed to reflect a narrow band of wavelengths when illuminated by a broadband light source (Cunningham et al., 2002). The adsorption of biomolecules on the sensor surface results in an increase in the dielectric permittivity of material in an evanescent electromagnetic field region in the media within ~200 nm of the surface (Arakawa and Kita, 1999), which in turn causes the reflected peak wavelength value (PWV) to shift to a greater value; thus, providing a simple mechanism for biomolecule detection. Unlike ELISAs that use a colorimetric reaction between an enzyme and a substrate to measure analyte concentrations (Voller et al., 1978), and RIAs that determine analyte concentrations based on the change of radioactivity of analyte samples (Marcus and Zinberg, 1975), PC biosensors utilize simple

Abbreviations: Ab, antibody; BIND, Biomolecular Interaction Detection; CI, confidence interval; FpAb, functionalized pAb; IONPs, iron-oxide nanoparticles; LOA, limit of agreement; LOD, limit of detection; mAb, monoclonal Ab; pAb, polyclonal Ab; PC, photonic crystal

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optics and intrinsic physical properties of the analyte as the mechanism of detection. PC detection systems have various diagnostic and screening applications in DNA microarrays (Mathias et al., 2010), cancer cell analysis (Chan et al., 2007), virus detection (Pineda et al., 2009), and pharmaceutical drug screening (Heeres and Hergenrother, 2011). Furthermore, PC biosensors are inexpensively manufactured from plastic materials and incorporated into liquid handling formats such as microplates (Cunningham et al., 2004) and microscope slides (Gallegos et al., 2013) for single-use applications.

One goal of current biomedical and nanotechnology research is to develop biosensor applications for point-of-care diagnostics in field settings (Nash et al., 2012). To achieve field readiness, methods to improve biosensor sensitivity as well as to increase biosensor versatility are needed to detect physiological concentrations of analytes comparable to commercial ELISA and RIA tests, often on the order of 1.0–1000.0 ng/mL. Non-specific binding and inconsistencies in sensitivity due to the proteinaceous nature of complex matrices like whole blood, serum, and plasma limit analysis (Byrne and Diamond, 2006). Current studies aimed at practical applications using optical biosensors acknowledge the difficulty of selective analyte detection in serum (Chung et al., 2005; Kumbhat et al., 2010; Kyprianou et al., 2013) and whole blood (Bonanno and DeLouise, 2007) necessary for point-of-care applications. A promising amplification approach using IONPs has shown to increase the signal-to-noise ratio in surface plasmon resonance optical biosensors in a chocolate bar matrix (Pollet et al., 2011) and serum and stool (Soelberg et al., 2009) matrices. Yet, to our knowledge, no studies with PC optical biosensors have tested IONPs as a method to enhance analyte detection in serum.

In the present work we describe proof-of-concept studies using IONPs to enhance sensitivity for detection of ferritin, a biomarker of iron deficiency anemia, in control serum and quality control samples using a PC biosensor.

2. Materials and methods

2.1. Reagents

3-Glycidoxypropyltrimethoxysilane (GTPMS), NaOH, Tween 20, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, and phosphate buffer saline (PBS) and StartingBlock blocking buffer from Pierce (Thermo Fisher Scientific). Double deionized water (DDW) was used in all experiments.

2.2. ELISA kits, antigen and antibodies (Ab)

Human ferritin ELISA kits were purchased from BioVendor (RCAN-F-4280R), GenWay Biotech, Inc. (GenWay; GWB-F4BE8D), and RayBiotech, Inc. (RayBiotech; ELH-Ferritin-001). Human liver ferritin (US Biological; F4015-21A) and Liquichek™ serum controls (Bio-Rad Laboratories) were dissolved in PBS or BSA to develop standard curves for the BIND and ELISA detection platforms, and act as quality controls for agreement and recovery studies. The monoclonal mouse anti-human liver ferritin Ab (mAb), used as the capture antibody in the BIND assay, were purchased from US Biological (F4015). As the detection Ab in the BIND, polyclonal goat anti-human liver ferritin Ab (pAb) were purchased from US Biological (F4015-17).

2.3. Iron-oxide nanoparticles conjugation protocol

Detection pAb were functionalized (FpAb) to iron-oxide nanoparticles (30 nm) as reported by vendor (Ocean NanoTech, LLC). Aliquots of 0.2 mL of IONPs were combined with 0.1 mL of Activation Buffer. Then, 100 μL of a solution containing 2 mg/ml EDAC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) and 1 mg/ml NHS (sulfo-N-hydroxysuccinimide) was added to the IONPs, mixed, and left at room temperature for 5–10 min with continual stirring. The next step mixed 0.4 mL of Coupling Buffer to the activated IONPs and then added at least 1 mg of pAb. Reaction time for conjugation lasted 2 h with continual mixing. Next, 10 μL of Quenching Buffer was left for 10 min at room temperature and then the entire mixture was transferred to a plastic cuvette and 3 mL of Wash/Storage Buffer was added. The cuvette was inserted into a SuperMag Separator™ (Ocean NanoTech, LLC) magnetic separator to allow conjugated magnetic FpAb to separate for 5 h. Liquids were aspirated without taking magnetic contents before FpAb were re-suspended in 3 mL and the last two steps were repeated for higher extraction. Conjugation was verified by gel electrophoresis tests (data not shown).

2.4. PC biosensor and readout system

As described and illustrated in Cunningham et al., 2004, the PC biosensors used in this work are comprised of a plastic replica molded periodic linear grating surface structure that is overcoated with a high refractive index TiO_2 thin film to create a resonant reflection surface that functions as a high efficiency reflector for only a narrow band of wavelengths near $\lambda = 855$ nm when covered with aqueous media. At the resonant wavelength, an optical standing wave is established at the PC surface. Adsorption of biomolecules or iron oxide tags, which have dielectric permittivity that is greater than water, results in displacement of water from the evanescent field region and an increase in the effective refractive index experienced by the optical standing wave. In turn, the augmented refractive index results in an increase in the resonant reflected wavelength from the PC. The PC biosensor structure is fabricated on sheets of plastic film and attached to bottomless standard format microplates. The PWV of the resonant reflection is measured by illuminating the PC at normal incidence with a broadband light source, and measuring the resonantly reflected wavelength with the aid of a spectrometer. Changes in PWV induced by adsorption of biomolecules can be monitored in each well, where the magnitude of the PWV shift can be used to quantify the amount of adsorbed material.

PC microplates (384-well) were purchased from SRU Biosystems, Inc. The Biomolecular Interaction Detection system (BIND; SRU Biosystems, Inc.) was used to measure interactions of ferritin with antibodies. The BIND illuminates the microplate with a broadband light source (λ range 400–700 nm) via an optical fiber positioned below the biosensor microplate. The system contains 8 parallel readout heads, and is capable of measuring the PWV of all 384-well biosensor microplate in ~10 s. The microplate may be re-scanned at preset intervals to generate kinetic plots of biomolecular binding. A ~2 mm diameter region of the biosensor is illuminated. A second parallel optical fiber is bundled with each illuminating fiber to capture reflected light, which is directed into a spectrophotometer. Detailed description of the design and operation of the BIND instrument can be found elsewhere (Cunningham et al., 2002, 2004).

2.5. PC detection procedure

2.5.1. Epoxy-silanization of PC biosensor surface

A 0.1 M NaOH solution was dispensed (20-μL) into the wells of a 384-well biosensor microplate and left to incubate for 1 h at room temperature (23 °C). After incubation, plates were ultra-sonicated (Fisher Scientific Isotemp202 Heater Ultrasonic bath) for 15 min. Wells were then aspirated and dried under nitrogen stream. Next the plate was placed in an oxygen plasma (Planar Plasma System, Texas Instruments Inc.) for 5 min. Then, 2.5% 3-glycidoxypropyltrimethoxysilane and 10 mM acetic acid solution in ethanol of was