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Giant magnetoresistive sensor array for sensitive and specific multiplexed food allergen detection

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ABSTRACT

Current common allergen detection methods, including enzyme-linked immunosorbent assays (ELISAs) and dip-stick methods, do not provide adequate levels of sensitivity and specificity for at-risk allergic patients. A method for performing highly sensitive and specific detection of multiple food allergens is thus imperative as food allergies are becoming increasingly recognized as a major healthcare concern, affecting an estimated 4% of the total population. We demonstrate first instance of sensitive and specific multiplexed detection of major peanut allergens Ara h 1 and Ara h 2, and wheat allergen Gliadin using giant magnetoresistive (GMR) sensor arrays. Commercialized ELISA kits for Ara h 1 and Ara h 2 report limits of detection (LODs) at 31.5 ng/mL and 0.2 ng/mL, respectively. In addition, the 96-well-based ELISA developed in-house for Gliadin was found to have a LOD of 40 ng/mL. Our multiplexed GMR-based assay demonstrates the ability to perform all three assays on the same chip specifically and with sensitivities at LODs about an order of magnitude lower than those of 96-well-based ELISAs. LODs of GMR-based assays developed for Ara h 1, Ara h 2, and Gliadin were 7.0 ng/mL, 0.2 ng/mL, and 1.5 ng/mL, respectively, with little to no cross-reactivity. These LODs are clinically important as some patients could react strongly against such low allergen levels. Given the limitations of current industrial detection technology, multiplexed GMR-based assays provide a method for highly sensitive and specific simultaneous detection of any combination of food-product allergens, thus protecting allergic patients from life-threatening events, including anaphylaxis, by unintentional consumption.

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1. Introduction

Food allergies are becoming increasingly recognized as a major primary healthcare concern, affecting an estimated 4% of the total population (Sicherer, 2011). In recent years, the prevalence of food allergies in infants and children has been reported to be 8% (Gupta et al., 2011; Sicherer, 2011) and is rising rapidly upwards towards 15–20% (Ho et al., 2014). The eight major allergens are peanuts, wheat, eggs, milk, soy, tree nuts, fish, and shellfish (U.S. Food and Drug Administration, 2015a). Peanut allergy prevalence has already doubled in a five-year time span (van Hengel, 2007). Consequently, there is an increasing concern and need to protect food allergic consumers from acute and potentially life-threatening allergic reactions through detection of trace contamination and accurate food labeling. In 2004, the Food Allergen Labeling and Consumer Protection Act (FALCPA) was passed mandating food manufacturers to label foods with any major food allergens (U.S. Food and Drug Administration, 2014). Despite the careful effort put forth by manufacturers, food allergic patients are still at high risk of consuming unintentional trace amounts of allergens that may have contaminated the food product at some point along the production line. Food products labeled with “may contain” limit food allergic consumers who completely avoid such labeled foods for safety concerns (Taylor and Baumert, 2010).

Current common trace allergen detection methods, including enzyme-linked immunosorbent assays (ELISAs) and dip-stick methods (Poms et al., 2005; Schubert-Ullrich et al., 2009; van Hengel, 2007), do not provide adequate levels of sensitivity and specificity for allergic patients at risk. Commercially available kits are limited to providing only qualitative or semi-quantitative information with significant false-positive rates due to cross-reactivity in complex food matrices (van Hengel, 2007). The lowest reported limit of detection (LOD) in such kits remains at the 0.1 mg/kg (ppm) level (Poms et al., 2005), inadequate for sensitive

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patients who may incur strong anaphylactic reactions to allergens at the ng/kg level. Given the limitations of current industrial detection technology technologies, a method for highly sensitive and specific simultaneous detection of multiple food-product allergens would protect allergic patients from life-threatening events, including anaphylaxis, by unintentional consumption. Furthermore, strongly accurate sensors of such allergens would greatly improve patient quality of life by providing confidence and peace-of-mind while enjoying foods.

We demonstrate first use of giant magnetoresistive (GMR) sensor arrays for sensitive and specific simultaneous quantification and detection of multiple allergens. GMR sensors operate on the principle of localized proximity magnetic sensing, whereby binding of a magnetically labeled biomolecule to an immunoassay structure on the GMR sensor surface generates a localized magnetic field that changes the resistance of the sensor. GMR sensors are based on nanostructured multilayer materials comprising of a noble metal spacer layer sandwiched between two ferromagnetic layers. GMR effect results from spin-dependent scattering. As electrons pass through the sensor sandwich structure, they are scattered in a fashion dependent on the orientation of the ferromagnetic layers. If the layers are oriented in a parallel manner, the electrons experience less scattering and resistance is therefore low. If the layers are oriented in an antiparallel manner, the electrons experience more scattering and resistance is therefore high. Our GMR sensors are of the spin valve (SV) type. In SV-GMR sensors, the top ferromagnetic layer is a “free” layer in which its magnetization direction can be easily changed with an applied magnetic field. The bottom ferromagnetic layer is a “fixed” layer in which its magnetization direction is held in place. At the quiescent point, the magnetizations of the free and fixed layers are orthogonal to each other. In the presence of an external magnetic field, the magnetization of the top free layer is changed to align parallel or antiparallel to the fixed layer, depending on the direction and magnitude of the external field. As magnetic nanoparticles in solution get captured on the sensor surface, the local magnetic field generated by the nanoparticles causes yet another change in the magnetization of the free layer. This change in magnetization produces a change in electrical resistance of the sensor in real-time, and is correlated to the concentration of a particular allergen in the sample (Chappert et al., 2007; Gaster et al., 2011b; Li et al., 2006).

Our lab has developed GMR sensor arrays that demonstrate sensitivities down to pg/mL ranges, excellent for trace allergen contaminant detection and quantification. Such sensitivities are also clinically important to patients who display strong allergic responses against allergen concentrations as low as ng/mL ranges. Because food samples, and other biological samples in general, lack detectable magnetic contents, the GMR sensing platform is enabled with low background signal levels, contributing to lower LODs (Hall et al., 2010). In addition to high sensitivity, our sensor arrays demonstrate multiplexed capabilities with low cross-reactivity that enable higher specificity and better quantification of any one or more allergen proteins in food samples with a single assay on a single chip (Gaster et al., 2011a; Osterfeld et al., 2008). Furthermore, the sensors can be mass produced, making them low cost and disposable (Hall et al., 2010). Other groups have also shown that GMR sensors could be applicable to a myriad of biomedical applications (Baselt et al., 1998; Graham et al., 2004; Sandhu, 2007; Schotter et al., 2004; Wang et al., 2014). With the multitude of advantages, GMR sensor arrays provide food manufacturers and allergic patients or caretakers with an attractive solution to the need for highly sensitive and specific detection of any multiple trace allergen contaminants in food samples.

2. Materials and methods

2.1. Reagents

All reagents and antibodies used in this study were commercially available. Bovine serum albumin (BSA), and TWEEN® 20 were purchased from Sigma-Aldrich Inc. Phosphate buffer saline (PBS) was purchased from Invitrogen. Peanut allergen Ara h 1 ELISA kit (containing mouse (2C12) anti-Ara h 1 capture antibody, purified Ara h 1 standard, and biotinylated mouse (2F7) anti-Ara h 1 detection antibody), mouse (1C4) anti-Ara h 2 capture antibody, purified peanut allergen Ara h 2 standard, and biotinylated rabbit anti-Ara h 2 detection antibody were purchased from Indoor Biotechnologies Inc. Rabbit anti-wheat gliadin capture antibody, purified wheat gliadin protein, and biotinylated anti-wheat gliadin detection antibody were purchased from Fitzgerald Industries International. Streptavidin-conjugated magnetic microbeads were purchased from Miltenyi Biotec.

2.2. Functionalization of GMR sensors

Our chips are comprised of an array of 8×10 GMR sensors. All GMR chips are pretreated with surface chemistry that enables functionalization of capture antibodies onto the sensor surface, shown on the right in Fig. 1. Pretreatment of the sensor chips are performed using a method previously detailed by Gaster et al. Briefly, the surface is cleaned with acetone, methanol, and isopropanol, exposed to oxygen plasma, and baked at 150 °C. A 10% solution of 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide hydrochloride and a 10% solution of N-hydroxysuccinimide is then added to the sensor surface and incubated at room temperature (Gaster et al., 2011b). Capture antibodies for Ara h 1, Ara h 2, and Gliadin (each at 1 mg/mL) are spotted on dedicated sensors (about 1.2 nL) using a robotic spotter (Scienion Flexarrayer S5). Biotinylated BSA (Biotin-BSA; positive control) and BSA (negative control) are also spotted on dedicated sensors. Spotted chips are placed inside a humid chamber and stored overnight at 4 °C. All sensors are fabricated with a top layer of corrosion resistant Tantalum along with a thin tri-oxide ($SiO_2/Si_3N_4/SiO_2$) passivation layer to protect the underlying GMR sensor from potential biological or chemical corrosions (Hall et al., 2010; Osterfeld et al., 2008).

2.3. GMR-based food allergen detection

GMR sensor array chips are removed from the humidity chamber and placed inside a tightly sealed cartridge. The sensor surface is washed using wash buffer (0.1% BSA in 0.05% TWEEN® 20 in PBS). The sensor surface is then blocked using 1% BSA for 1 h at room temperature to prevent non-specific binding and then washed using wash buffer. A mixture of Ara h 1, Ara h 2, and Gliadin standard is added and allowed to incubate for 1 h at room temperature during which the allergen of interest binds to the corresponding capture antibody on the sensor surface. A wash step follows and biotinylated detection antibody (each at 1 μg/mL) is added and incubated for 1 h at room temperature. For cross-reactivity tests, a single detection antibody is added. One chip is dedicated to only anti-Ara h 1 detection antibody, one chip to only anti-Ara h 2 detection antibody, and one chip to only anti-Gliadin detection antibody. For multiplexed assays, a mixture of anti-Ara h 1, anti-Ara h 2, and anti-Gliadin detection antibodies is added. Biotinylated detection antibody binds to the respective allergen of interest. After a final wash step, the GMR sensor chip is plugged into as data acquisition system and exposed to an external magnetic field through a magnetic coil. The data collection software is started and streptavidin magnetic nanoparticles (50 μL) are added onto the sensor chip surface. Real-time changes in resistance for