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Low-fouling surface plasmon resonance biosensor for multi-step detection of foodborne bacterial pathogens in complex food samples

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ABSTRACT

Recent outbreaks of foodborne illnesses have shown that foodborne bacterial pathogens present a significant threat to public health, resulting in an increased need for technologies capable of fast and reliable screening of food commodities. The optimal method of pathogen detection in foods should: (i) be rapid, specific, and sensitive; (ii) require minimum sample preparation; and (iii) be robust and cost-effective, thus enabling use in the field. Here we report the use of a SPR biosensor based on ultra-low fouling and functionalizable poly(carboxybetaine acrylamide) (pCBAA) brushes for the rapid and sensitive detection of bacterial pathogens in crude food samples utilizing a three-step detection assay. We studied both the surface resistance to fouling and the functional capabilities of these brushes with respect to each step of the assay, namely: (I) incubation of the sensor with crude food samples, resulting in the capture of bacteria by antibodies immobilized to the pCBAA coating, (II) binding of secondary biotinylated antibody (Ab_2) to previously captured bacteria, and (III) binding of streptavidin-coated gold nanoparticles to the biotinylated Ab_2 in order to enhance the sensor response. We also investigated the effects of the brush thickness on the biorecognition capabilities of the gold-grafted functionalized pCBAA coatings. We demonstrate that pCBAA-compared to standard low-fouling OEG-based alkanethiolate self-assemabled monolayers-exhibits superior surface resistance regarding both fouling from complex food samples as well as the non-specific binding of S-AuNPs. We further demonstrate that a SPR biosensor based on a pCBAA brush with a thickness as low as 20 nm was capable of detecting *E. coli* O157:H7 and *Salmonella* sp. in complex hamburger and cucumber samples with extraordinary sensitivity and specificity. The limits of detection for the two bacteria in cucumber and hamburger extracts were determined to be 57 CFU/mL and 17 CFU/mL for *E. coli* and 7.4×10^3 CFU/mL and 11.7×10^3 CFU/mL for *Salmonella sp.*, respectively. In addition, we demonstrate the simultaneous detection of *E. coli* and *Salmonella sp.* in hamburger sample using a multichannel SPR biosensor having appropriate functional coatings.

1. Introduction

Outbreaks of foodborne illnesses have shown that foodborne bacterial pathogens present a significant threat to public health (CDC, 2014, EFSA, 2014). Recent cases include the discovery of *Staphylococcus aureus* in ice cream (Fetsch et al., 2014), *Escherichia coli* O157:H7 in spinach and ground beef (Heaton and Jones, 2008), *Salmonella* in peanut butter (Gerner-Smidt et al., 2007), and *Listeria monocytogenes* in ready-to-eat meats (Gerner-Smidt et al., 2007). The CDC estimates that every year in the US over 48 million people are infected, resulting in 128,000 hospitalizations and 3000 deaths (CDC, 2014). Infectious doses for pathogens such as *E. coli* O157:H7 and *Salmonella* are not strictly defined and depend on the host age and health (Food and Drug Administration, 2012, Paton and Paton, 1998). Some of the published data indicates that they may be as low as 1 to 100 organisms (Food and Drug Administration, 2012, Paton and Paton, 1998), while other studies report much higher infective doses (Food and Drug Administration, 2012, Kothary and Babu, 2001). The most commonly infected foods include dairy products, beef, poultry, vegetables, and drinking water. These outbreaks demonstrate an urgent need for an improvement in the screening and diagnosis of food commodities.

Culture and colony counting methods represent the most

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frequent approaches for the detection of foodborne bacterial pathogens in standardized laboratories; however, these methods are excessively time consuming and have been reported to have limited sensitivity to diverse bacterial pathogens (Gracias and McKillip, 2004, Lazcka et al., 2007). In order to improve both sensitivity and selectivity, these approaches can be combined with polymerase chain reaction (PCR)-based techniques (Lampel et al., 2000, Li and Mustapha, 2004, Rodriguez-Lazaro et al., 2005, Simpson and Lim, 2005). Although PCR methods possess relatively high sensitivity (with LODs as low as a few CFU/mL), they require expensive equipment and are likewise time-consuming (Postollec et al., 2011). Currently, the most advanced techniques to detect bacterial pathogens in food and environmental samples are represented by both immunoassay-based methods as well as DNA and fluorescent microarrays (Call et al., 2003, Cudjoe et al., 1995, Disney and Seeberger, 2004, Mansfield and Forsythe, 2001, Yu et al., 2002). These methods are capable of relatively fast detection with respect to culture-based methods, yet they also require trained personnel and expensive equipment.

Biosensors have been shown to provide a simple, cost-effective alternative to conventional methods for the detection of foodborne pathogens and furthermore, are expected to enable the field detection of foodborne pathogens, thus providing significant decreases in the overall analysis time. To date, a variety of biosensors for the detection of bacteria have been demonstrated. These include electrical biosensors based on amperometry (Abdel-Hamid et al., 1999, Li et al., 2012, Lin et al., 2008, Perez et al., 1998) or electrochemical impedance spectroscopy (Barreiros dos Santos et al., 2013, Yang et al., 2004) as well as optical biosensors employing fluorescent labels (Ko and Grant, 2006, Rohde et al., 2015, Sanvicens et al., 2011, Yang and Li, 2006, Zordan et al., 2009) or label-free optical methods (Baccar et al., 2010, Cho et al., 2015, Linman et al., 2010, Rodriguez-Emmenegger et al., 2011a, Tawil et al., 2012, Wang et al., 2011, 2012).

Surface plasmon resonance (SPR) affinity biosensors represent the foremost label-free optical biosensor technology regarding the detection of foodborne pathogens. Typical LODs for bacteria achieved by SPR biosensors are on the order of 10^4 CFU/mL or higher for direct detection, where no preconcentration or amplification steps are employed. A lower limit of detection was demonstrated by Yazgan et al. (2014) who reported the detection of *E. coli* in buffer with a LOD of 2.5 CFU/mL through the use of modified carbohydrates as bioreceptors. Another strategy for improving LODs has been demonstrated by Torun et al., who combined an SPR biosensor with immunomagnetic separation and reported the detection of *E.coli* in buffer with a LOD of 3 CFU/mL. They also applied their method to the detection of *E.coli* in moderately complex samples (lake, river, and tap water samples); limits of detection for these matrices were not reported (Torun et al., 2012). Bouguelia et al. (2013) reported the SPR detection of various bacterial pathogens with LODs as low as ~3 CFU/mL in matrixes composed of the addition of crude food samples to growth medium. In this sensor a growth chamber was situated directly on the SPR surface, where at a temperature of 37 °C they were able to monitor the growth of each pathogen in real-time; however, the detection of lower pathogen concentrations required analysis times of up to 10 h.

One of the key challenges hindering the application of SPR biosensors for the detection of bacterial pathogens in real-world complex food samples remains interfering effects stemming from the sample matrix, particularly the non-specific adsorption of non-target molecules from the sample to the sensing surface (causing a false positive signal). Correspondingly, a number of research groups have pursued the development of advanced functional coatings in order to suppress this effect. An optimum functional coating for the biosensor-based analysis of food samples is one that combines a high number of functional bioreceptors with an ability to resist fouling from complex media (Homola, 2008, Vaisocherova et al., 2015a). Among the wide variety of previously reported low-fouling coatings, zwitterionic polymeric coatings have been recently demonstrated as a promising functionalizable low-fouling surface for label-free biosensing in complex media (Brault et al., 2010, Vaisocherova et al., 2015a, 2008). Nevertheless, the biosensor capabilities of such coatings have been primarily demonstrated in complex biological fluids (blood plasma or serum) (Banerjee et al., 2011, Vaisocherova et al., 2008); the use of these coatings has never been explored with food samples. Compared to blood plasma samples, food commodities contain diverse sets of components including lipids, proteins, saccharides, water, vitamins, minerals, and even synthetic low-molecular-weight additives with variable contents. This complexity results in diverse food sample physico-chemical properties (Sikorski, 2002), thus the design of a robust, functional, low-fouling coating for reliable biosensing in crude food samples remains challenging.

In this work, we present the first use of ultra-low fouling, functionalizable poly(carboxybetaine acrylamide) (pCBAA) brushes as a unique sensing surface for the multi-step detection of bacterial pathogens in crude hamburger and cucumber samples. We studied both the functional as well as the surface resistance capabilities of the pCBAA coatings for each step of a detection assay that included: (I.) the capture of *E. coli* O157:H7 and *Salmonella* sp. in hamburger and cucumber samples, (II.) the capture of biotinylated secondary antibody, and (III.) the signal enhancement using streptavidin-coated gold nanoparticles. We compare our results to those using a standard low-fouling carboxy-functional OEG-based AT-SAMs, and show that the pCBAA brushes have similar levels of functionalization while providing over an order of magnitude improvement in fouling resistance. Finally, we demonstrate the effectiveness of these brushes via the use of a pCBAA-based SPR biosensor for the sensitive and specific detection of low levels of *E. coli* O157:H7 and *Salmonella* sp. in complex cucumber and hamburger samples.

2. Materials and methods

2.1. Reagents and biological materials

N-hydroxysuccinimide (NHS) and *N*-ethyl-*N*′-(3-diethylaminopropyl) (EDC) were purchased from PharmaTech, Czech Republic. Ethanol (purity ≥99.9%) was purchased from Merck, USA. The buffer solutions were prepared using Millipore Q water (18.0 MΩ. cm). Phosphate buffered saline (PBS, 0.01 M phosphate, 0.138 M sodium chloride, 0.0027 M potassium chloride, pH 7.4 at 25 °C) was prepared from a stock solution purchased from Sigma-Aldrich, Czech Republic. Tween® 20 (Tween), ethanolamine (EA), and casein blocking buffer (casein) were purchased from Sigma-Aldrich, Czech Republic. 10 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid, pH 8.0 at 25 °C (HEPES) and 10 mM sodium acetate buffer, pH 5.0 at 25 °C (SA) were prepared from stock solutions purchased from Sigma Aldrich, Czech Republic. The bromine-terminated alkanethiol (HS-C_{11}-OC(O)-IzoButyrate-Br), HS-C_{11}-$(EG)_4$-OH, and HS-C_{11}-EG_6-OCH_2-COOH thiols were purchased from Prochimia, Poland. The carboxybetaine acrylamide monomer (CBAA) was purchased from Zwitter Technology, Seattle, USA. The CuBr, $CuBr_2$, 2,2′-dipyridyl (BiPy), were purchased from Sigma-Aldrich, Czech Republic. Tetrahydrofuran (THF, purity ≥99.9%) was purchased from Penta, Czech Republic.

Heat-killed *Escherichia coli* O157:H7 (*E. coli*), *Salmonella sp.* (*Salmonella*), primary antibodies (Ab_1), and biotinylated secondary antibodies (Ab_2) against *E. coli* and *Salmonella* (anti-*E.coli*, b-anti-*E. coli*, anti-*Salmonella*, b-anti-*Salmonella*) were purchased from KPL, Inc., USA.