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Rapid and label-free detection of protein a by aptamer-tethered porous silicon nanostructures

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ABSTRACT

Protein A, which is secreted by and displayed on the cell membrane of *Staphylococcus aureus* is an important biomarker for *S. aureus*. Thus, its rapid and specific detection may facilitate the pathogen identification and initiation of proper treatment. Herein, we present a simple, label-free and rapid optical biosensor enabling specific detection of protein A. Protein A-binding aptamer serves as the capture probe and is immobilized onto a nanostructured porous silicon thin film, which serves as the optical transducer element. We demonstrate high sensitivity of the biosensor with a linear detection range between 8 and 23 μM. The apparent dissociation constant was determined as 13.98 μM and the LoD is 3.17 μM. Harnessing the affinity between protein A and antibodies, a sandwich assay format was developed to amplify the optical signal associated with protein A capture by the aptamer. Using this approach, we increase the sensitivity of the biosensor, resulting in a three times lower LoD.

1. Introduction

Staphylococcus aureus is a leading cause of infections and bacteremia in humans, but also causing food-borne diseases due to excretion of several enterotoxins (Foster et al., 2015). Treatment is especially challenging as *S. aureus* has a remarkable ability to rapidly adapt its genetic characteristics and develop resistance against new antibiotics (Maple et al., 1989; Chambers and DeLeo, 2009). Hence, the most efficient way to fight *S. aureus* infections is to prevent their transmission by isolation, decontamination and strict hygienic guidelines. Moreover, studies have concluded that active screening for methicillin-resistant *Staphylococcus aureus* (MRSA) can efficiently reduce bloodstream infections, which results in high mortality (Tacconelli et al., 2009; Pronovost et al., 2006).

Rapid and reliable detection and identification of MRSA is critical for effective infection control as well as for therapeutic decisions. Traditional culturing techniques using selective media are sensitive and cost-effective, but are time-consuming (several days) and therefore problematic (Lee et al., 2013; Francois et al., 2003). Molecular methods based on polymerase chain reaction (PCR) exhibit faster turnaround times (~2–6 h) and are considered the gold standard for MRSA detection nowadays; however, they are cost-intense, require trained staff and may not keep up with new evolving genetic variants (Kluytmans, 2017). A rapid user-friendly point-of-care test for *S. aureus* is thus urgently needed to allow fast screening of patient samples and provide effective infection control.

Protein A, a virulence factor specific for *S. aureus*, is linked to peptidoglycans on the bacterial cell surface and promotes general surface adhesion (DeDent et al., 2007). Protein A binds to the von Willebrand factor (vWF), an essential protein for hemostasis, and therefore promotes wound infection. It also binds the Fc-region of human antibodies, thereby inhibiting phagocytosis, which in consequence prevents bacterial elimination (Hartleib et al., 2000; Kim et al., 2012). Real-time PCR for typing and detection of different *S. aureus* variants utilizes the specific spa gene, encoding protein A. As the gene is highly conserved, showing one mutation in 70 months (Kahl et al., 2005), protein A provides an optimal molecular marker for the detection of *S. aureus*. Several assays, mostly ELISA (Enzyme Linked Immunosorbent Assay) based, for the detection of protein A using antibodies have been developed (Hsin Chang et al., 1996) and are commercially available. While these labeled techniques are highly sensitive, they suffer from several limitations,

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which are ascribed to the complicated required equipment and the delicate nature of the antibody receptor and its costs (Song et al., 2012; Lakhin et al., 2013).

Aptamers are single-stranded DNA or RNA oligonucleotides with the ability to specifically bind their target due to their unique 3-dimensional structure. Aptamers are selected *in vitro* for a specific target using the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) process (Tuerk and Gold, 1990). Aptamers can be engineered towards specific targets, ranging from small molecules or proteins to whole cells, and synthesized with high reproducibility and at a fraction of the cost of antibodies (Song et al., 2012; Bunka and Stockley, 2006; Strehlitz et al., 2008). Thus, due to these advantageous properties, a variety of aptamer-based analytical methods and biosensors have been developed in recent years, also for the detection of *S. aureus* (Yuan et al., 2014; Duan et al., 2012; Jia et al., 2014; Baumstummler et al., 2014). Lian et al. developed a piezoelectric sensor employing *S. aureus* aptamer and demonstrated detection at bacterial concentrations as low as 59 CFU/mL in milk samples (Lian et al., 2015). The aptamer they used was selected against *S. aureus* whole cells and as such, the molecular binding site of the aptamer on the cell surface is unknown (Shangguan et al., 2015). Since *S. aureus* has many variants and is evolving quickly, the aptamer may lose its affinity, thereby limiting the applicability of this method (Kahl et al., 2005).

Recently, the Strehlitz group has developed a modified SELEX process, termed FluMag-SELEX, wherein the target is immobilized on magnetic beads and fluorescent labels are used for aptamer quantification (Stoltenburg et al., 2005). Using this process, they selected an aptamer binding protein A with high affinity and demonstrated that it binds specifically to both, native and recombinant protein A, but not to other immunoglobulin-binding proteins like protein G.

Herein, we use this aptamer as a receptor for the design of a label-free optical biosensor. The biosensor is based on a porous silicon (PSi) nanostructure which is used as the optical transducer. PSi-based optical biosensors have demonstrated outstanding performance in terms of rapid and reliable detection of numerous targets (Massad-Ivanir et al., 2011; Dancil et al., 1999; Bonanno and DeLouise, 2007; Beavers et al., 2014). Specifically, biosensors employing interferometric Fourier transform spectroscopy (RIFTS) (Sailor, 2017; Pacholski et al., 2006a; Pacholski et al., 2005), which harness the series of Fabry-Pérot interference fringes from light reflections from the top and bottom interfaces of a porous thin film, allow for the design of simple and sensitive detection of a specific analyte upon its binding to surface-tethered capture probes (Jane et al., 2009; De Stefano et al., 2007; Jane et al., 2007). Recently, the excellent integration of aptamers as capture probes with PSi-based transducers has been demonstrated, enabling exceptionally stable and reliable biosensing, applicable for both, proteins and bacteria (Urmann et al., 2015; Urmann et al., 2016; Yoo et al., 2013; Terracciano et al., 2016).

In the present study, protein A-targeting aptamers are conjugated to PSi thin films and the resulting biosensors demonstrate a specific detection and quantification of protein A in a range of 2–50 μM with a total assay time of <2 h. The biosensing scheme is further optimized and we show a measured limit of detection (LoD) of 1 μM.

2. Material and methods

2.1. Materials

Heavily boron doped p-type Si wafers (0.0008 Ω cm resistivity, <100>-oriented) were purchased from Sil'tronix Silicon Technologies (France). Aqueous HF (48%) and ethanol absolute were obtained from Merck and toluene and acetone were supplied by Gadot Biochemical Industries LTD (Israel). (3-aminopropyl)-triethoxysilane (APTES), succinic acid, *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC) and all buffer salts were purchased from Sigma-Aldrich Chemicals. Buffers were prepared with deionized water (18.2 MΩ cm), filtered and autoclaved prior to use. The protein A-binding aptamer, selected by Stoltenburg et al. (2015) was used in its truncated form PA#2/8[S1-58]: 5′- ATA CCA GCT TAT TCA ATT AGC AAC ATG AGG GGG ATA GAG GGG GTG GGT TCT CTC GGC T – 3′(abbreviated as PAA). Its selection buffer (SB) was composed of 20 mM Tris base (Trizma, Sigma Aldrich), 100 mM NaCl, 5 mM KCl, 10 mM $MgCl_2$, and 1 mM $CaCl_2$. MES buffer was composed of 100 mM 2-Morpholinoethanesulfonic acid at pH 6. PAA and a completely randomized 40 nucleotides long DNA-Pool (N_{40}) were purchased with a 3′-amino-C6- modification from Integrated DNA Technologies (Coralville, USA). Biosensing experiments were performed with the following proteins: recombinant protein A (PA) and IgG from human serum (both from Sigma-Aldrich Chemicals). As Tween 20 enhances protein solubility, all proteins solutions were prepared in SB supplemented with 0.005% Tween 20 (SBT).

2.2. Preparation of oxidized PSi

Si wafers were anodized in a two-step process. First, a sacrificial layer was etched at a constant current density of 375 mA/cm^2 for 30 s in a solution of 3:1 (v/v) aqueous HF (48%) and ethanol. Etching setup details are reported elsewhere (Sailor, 2017; Shtenberg et al., 2012). The resulting PSi layer was dissolved in an aqueous NaOH solution (0.1 M). Next, a second etching step was performed using the above-mentioned conditions. After each step, the silicon surface was thoroughly rinsed with ethanol and dried under a stream of nitrogen. Finally, the PSi samples were thermally oxidized in a tube furnace (Thermolyne) at 800 °C for 1 h in ambient air, yielding oxidized PSi ($PSiO_2$) scaffolds.

2.3. Characterization of $PSiO_2$ films

The nanostructure and the thickness of the neat $PSiO_2$ samples were studied by a Carl Zeiss Ultra Plus high-resolution scanning electron microscopy (HRSEM) at an accelerating voltage of 1 keV. The porosity of the films was characterized by gravimetry (for porosity), and the spectroscopic liquid infiltration method (SLIM) (Sailor, 2017), as described by Massad-Ivanir et al. (2010).

2.4. Aptamer immobilization onto $PSiO_2$ films

The aptamer was conjugated to the $PSiO_2$ films by a previously described coupling chemistry (Urmann et al., 2015). Briefly, the $PSiO_2$ was amino-modified by incubation (1 h) in a solution of APTES in toluene (42 mM); after which, the samples were rinsed with toluene, ethanol and acetone and dried under a nitrogen stream. Next, the $PSiO_2$ was incubated for 30 min in a freshly prepared solution of 100 mg succinic acid in 4.7 mL DMSO (dimethyl sulfoxide) and 300 μL of 0.1 M $NaHCO_3$, pH 9.4 and subsequently washed with DMSO and deionized water. Successive chemical modifications were carried out in a custom made Plexiglas flow cell (Urmann et al., 2015). A 52 mM solution of EDC in MES was introduced into the flow cell and allowed to react for 1 h. Next, 50 μL of the aptamer solution (75 μM in MES) was introduced and incubated with the surface for 1 h, followed by washing with 10 mL MES. Finally, the surface was incubated for 30 min with 300 μL of 0.1 M ethanolamine solution to block remaining active sites.