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Rapid and low-cost biosensor for the detection of Staphylococcus aureus

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ABSTRACT

Staphylococcus aureus (S. aureus) is one of the most common etiological agents in hospital-acquired infections and food-borne illness. *S. aureus* toxins and virulence proteases often circulate in host blood vessels leading to life-threatening diseases. Standard identification approaches include bacterial culturing method, which takes several days. Other nucleic acid-based methods were expensive and required trained personnel. To surmount these limitations, a paper-based biosensor was developed. The sensing mechanism was based on the proteolytic activity of *S. aureus* proteases on a specific peptide substrate, sandwiched between magnetic nanobeads and gold surface on top of a paper support. An external magnet was fixed on the back of the sensor to accelerate the cleavage of the magnetic nanobeads-peptide moieties away from the sensor surface upon test sample dropping. The colour change resulting from the dissociation of the magnetic nanobeads moieties was detected by the naked eye and analysed using ImageJ analysis software for the purpose of quantitative measurement. Experimental results showed detection limits as low as 7, 40 and 100 CFU/mL for *S. aureus* in pure broth culture, and inoculated in food produces and environmental samples, respectively upon visual observation. The specificity of the sensor was examined by blind testing a panel of food-contaminating pathogens (*Listeria monocytogenesis* 19115 and *E. coli O157:H7*), clinical isolates (methicillin-resistant *S. aureus* (*MRSA*) and *Candida albicans*) and standard (*Pseudomonas aeruginosa* 15692) pathogens. Negative read-out was observed by the naked eye for all tested isolates except for MRSA. Moreover, this sensing tool requires minute's time to obtain the results. In conclusion, this sensing platform is a powerful tool for the detection of *S. aureus* as a potential point-of-care diagnostic platform in hospitals and for use by regulatory agencies for better control of health-risks associated with contaminated food consumption.

1. Introduction

Staphylococcus aureus (*S. aureus*) is a facultative anaerobic, gram-positive bacterium discovered by Dr. Alexander Ogston (Ogston, 1984). After which, *S. aureus* bacterium received a lot of attention, being allied with health care-associated infections (HAIs) (http://www.cdc.gov/HAI/organisms/staph.html). These infections were correlated with a number of risk factors, including long-time hospitalization, hospital-costs and mortality. On the other hand, *S. aureus* bacterium was among the top five pathogens associated with food-borne illnesses (http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html; Wang et al., 2011).

S aureus pathogens usually grow in open wounds and urinary tracts, leading to numerous ailments, from minor skin infections to life-threatening diseases such as abscesses (Kapral et al., 1980), pneumonia (Robertson et al., 1958), meningitis (Gordon et al., 1985), endocarditis (Fowler et al., 2007) and septicaemia (Cross et al., 1983). The National Institute of Health and Center for Disease Control and Prevention reported 94,000 life-threatening antibiotic-resistant infection cases out of which 500,000 people were infected with *S. aureus* pathogen in the United States of America annually (Klein et al., 2007).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common *S aureus* resistant strains in hospitals (Wang et al., 2011). MRSA infections always cause serious antibiotic resistant illnesses and high mortality (Cosgrove et al., 2003). Thus, to combat the spread of MRSA infections and to minimize the waste of public resources, new strategies for the development of a specific and feasible diagnostic biosensor for the detection of *S. aureus* is urgently needed.

It is worth mentioning that the *Staphylococcus* species is usually present in the nostrils and on the skin of warm-blooded animals, and thus can contaminate food products derived from animals such as meat, milk and eggs (Yang et al., 2011). Also, poor hygienic conditions by food handlers during manufacturing processes can contaminate

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foods (Goto et al., 2007). *S. aureus* bacterium can also live in harsh environments, and so, under temperature-abused conditions, it grows and produces virulence proteases in addition to other endotoxins, leading to staphylococcal food poisoning (SFP), which is marked by severe gastrointestinal symptoms such as emesis, diarrhea, and/or abdominal pain (Doyle MP et al., 2007). Recently, the Center for Disease Control and Prevention (CDC) estimated 240,000 illnesses with 1,000 hospitalizations and 6 deaths associated with SFP annually (Scallan et al., 2011).

Clearly, in case of food poisoning, specific *S. aureus* detection is important for proper treatment, but it is more important to prevent the occurrence of the disease. One way of doing so is to spot contaminated products and sites. For example, it is preferred to detect contaminated food products before reaching consumers. Conspicuously, this could be achieved through the development of cheap, stripe formate biosensors applicable at the retail level in order to protect the consumer.

Conventionally, *S. aureus* detection was based on bacterial culture methodology (Bocher et al., 2008). However, this process is time-consuming, labor-intensive and takes several days. This delays bacterium identification, which is unacceptable in emergency and critical illnesses such as sepsis, thus limiting its practical application for rapid diagnosis (Gilbert, 2002). Other ultra-sensitive detection methods were reported based on nucleic acid amplification, such as ligase chain reaction (LCR) (Moore and Curry, 1998), strand displacement amplification (SDA) (Edman et al., 2000) and polymerase chain reaction (PCR) (Cheng et al., 2006). Fortunately, these technologies were capable of detecting low numbers of bacterial cells, but took several hours. Moreover, these technologies were expensive and required prior bacterial DNA isolation, preparation of enzyme reaction mixtures and expensive instruments for nucleic acid amplification. Alternative methods such as antibody-based immunoassays and immuno-PCR assays were well-established and used extensively (Huang and Chang, 2004; Zhu et al., 2014). However, antibody-DNA conjugation and purification processes are tedious, in addition to the high costs of the instruments, which prevents them from being procured quickly. Chang et al. (2013) reported the development of a non-PCR–based method, which combines aptamer-conjugated gold nanoparticles and a resonance light-scattering detection system. This method successfully detects a single *S. aureus* cell within 1.5 h. However, this method needs sophisticated instruments. At present, nanotechnology's application in the development of biosensors-in particular, those capable of identifying protease activity as a disease biomarker, were very attractive due to their high specificity and sensitivity (Esseghaier et al., 2014; Suaifan et al., 2013a, 2013b; Suaifan et al., 2016a, 2016b; Wignarajah et al., 2015; Alhogail et al., 2016; Suaifan, 2016). However, expensive instruments are still required for these real-time detection methods. Therefore, there is a need for a "sample-to-answer", "hand-held", specific and sensitive diagnostic biosensor capable of detecting *S. aureus* proteases, as a biomarker for the presence/contamination of *S. aureus* in a short time.

Accordingly, this study aimed at the development of a magnetic nanobeads-peptide probe, which would be cleaved specifically by *S. aureus* virulence proteases. This probe would then be integrated with a gold sensing platform via Au-S linkage to provide specific and cost-effective strip-format diagnostic biosensor for the qualitative and quantitative detection of *S. aureus*. This biosensor would be label-free, cost-effective, quick, simple and can be read by the naked eye.

2. Experimental section

2.1. Materials and method

Carboxyl-terminated magnetic nanobeads (50 nm in diameter) were supplied by Turbo beads (Switzerland). *N*-Hydroxysuccinimide (NHS), 1-(3-Dimethylaminopropyl)-3-Ethyl-Carbodiimide (EDC), pH indicator strips were purchased from Sigma Aldrich (Dorset, UK). Self-adhesive Magnet sheets were purchased from Polarity Magnets Company (UK). The peptide sequence NH_2-Ahx-ETKVEENEAIQK-Ahx-Cys was synthesised by Pepmic Co., Ltd. (Suzhou, China). Self-adhesive tape was purchased from Whatman (London, UK). Brain Heart Infusion broth and agar were purchased from SDA Oxoid, Ltd. (Basingstoke, UK). Sterile filter 0.22 μm was from Millipore (UK). The wash/storage buffer (10 mM Tris base, 0.15 M sodium chloride, 0.1% (w/v) bovine serum albumin, 1 mM ethylenediaminetetraacetic acid, 0.1% sodium azide, pH 7.5) and the coupling buffer (10 mM potassium phosphate, 0.15 M sodium chloride, pH 5.5) were prepared from chemicals of analytical grade.

2.2. Bacterial strain culturing and protease preparation

S aureus (ATCC 25923), *Listeria* monocytogenes (ATCC 19115) and *Pseudomonas* aeruginosa (*P. aeruginosa*, ATCC 15692), purchased from Sigma Aldrich (UK) were streaked on BHI agar plate and incubated at 37.0 °C for 24 h. One colony was then isolated, inoculated into BHI broth and incubated overnight at 37 °C. The primary bacterial culture (PBC) was serially diluted to get the bacterial colony forming unit (CFU/mL) value of the original stocks by using viable count spread dilution method. Then, the bacteria were pelted by centrifugation at 3000Xg for 10 min. The supernatant was then filtered to obtain the crude protease solution of each bacterial concentration. The protease's proteolytic activity was measured by the universal, non-specific casien assay and was determined as the amount in micromoles of tyrosine equivalents released from casien per minute (Cupp-Enyard, 2008). An increase in *S. aureus* protease's proteolytic activity strength was correlated to bacterial culture concentration (colony forming unit (CFU/mL)).

2.3. Conjugation of S. aureus peptide substrates with magnetic-nanobeads

Magnetic-nanobeads suspension (1 mL) was mixed with the peptide substrate NH_2-Ahx-ETKVEENEAIQK-Ahx-Cys (1.0 mg/mL), coupling agent EDC (0.57 mg/mL) and NHS (12 μg/mL). The mixture was tremor gently at room temperature for 24 h. The uncoupled peptides were removed by washing the magnetic-nanobeads three times with a wash buffer. Finally, the beads were stored at 4 °C in a storage buffer. (Esseghaier et al., 2014; Suaifan et al., 2012; 2013a; 2013b).

2.4. Fabrication of the gold sensing platform

Schematic 1 shows the detailed steps for the preparation of the sensing platform. Self-adhesive tape was coated with a thin layer (30 nm) of gold which was sputtered using RF magnetron sputtering (Nordiko Ltd). Conditions 5mT pressure Argon gas. Power 100 W, 5 min at School of Engineering at Cranfield University. Following this, a rectangular piece (~1.5–2×3 mm) was stacked over the front face of the plastic physical support (Scheme 1A).

2.5. Immobilization of the sensing monolayer (SAM)

Magnetic nanobeads-peptide solution was placed over the gold sensing platform and allowed to dry at room temperature (Scheme 1B). Upon completion of the immobilization step, the sensor platform golden colour turns into black as observed by the naked eye. Following this, an external magnet (12.5×12.5×5 mm) with field strength of 573 gauss at a distance of 10 mm was passed over the sensor platform to remove any unattached magnetic nanobeads as shown in (Scheme 1C). Then, a round paper magnet was fixed on the back of the strip at around 5 mm distance beneath the gold sensor platform as shown in (Scheme 1D).