Biosensors and Bioelectronics 74 (2015) 335–340

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

SPR-DNA array for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in combination with loop-mediated isothermal amplification

Kawin Nawattanapaiboon [a], Wansika Kiatpathomchai [b], Pitak Santanirand [c], Apirom Vongsakulyanon [c], Ratthasart Amarit [d], Armote Somboonkaew [d], Boonsong Sutapun [e], Toemsak Srikhirin [a,f,*]

[a] Materials Science and Engineering Programme, Faculty of Science, Mahidol University, Rama VI Rd., Phayathai, Bangkok 10400, Thailand
[b] Bioengineering and Sensing Technology Laboratory, BIOTEC, National Science and Technology Development Agency (NSTDA), Pathum Thani 12120, Thailand
[c] Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Rama VI Rd., Phayathai, Bangkok 10400, Thailand
[d] Photonic Technology Laboratory, NECTEC, National Science and Technology Development Agency (NSTDA), Pathum Thani 12120, Thailand
[e] School of Electronic Engineering, Institute of Engineering, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand
[f] Physics Department, Faculty of Science, Mahidol University, Rama VI Rd., Phayathai, Bangkok 10400, Thailand

ARTICLE INFO

Article history:
Received 31 March 2015
Received in revised form
2 June 2015
Accepted 17 June 2015
Available online 25 June 2015

Keywords:
Surface plasmon resonance
Loop-mediated isothermal amplification
DNA array
DNA sensor
Methicillin-resistant *Staphylococcus aureus*
Clinical specimen

ABSTRACT

In this study, we evaluated surface plasmon resonance imaging (SPR imaging) as a DNA biosensor for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) which is one of the most common causes of nosocomial infections. The DNA sample were collected from clinical specimens, including sputum and blood hemoculture were undergone LAMP amplification for 0.18 kbp and 0.23 kbp DNA fragments of *fem*B and *mec*A genes, respectively. The self-assembled monolayer surface (SAMs) was used for immobilized streptavidin-biotinylated probes on the sensor surface for the detection of LAMP amplicons from MRSA. Both LAMP amplicons were simultaneously hybridized with ssDNA probes immobilized onto a bio-functionalized surface to detect specific targets in the multiplex DNA array platform. In addition, the sensor surface could be regenerated allowing at least five cycles of use with a shortened assay time. The detection limit of LAMP–SPR sensing was 10 copies/μl and LAMP–SPR sensing system showed a good selectivity toward the MRSA.

1. Introduction

According to a World Health Organization (WHO) reported in 2014, antimicrobial resistance has increased steadily and and remains uncontrolled in widespread areas. This is related to the ability of microorganisms to adapt and become resistant to antibiotic drugs. Antimicrobial resistance can be transferred by genetic elements such as plasmids, naked DNA or transposons that contain a drug resistance gene from one bacterium to another (Levy and Marshall, 2004). This is why many variations of resistance patterns spread with remarkable speed. Antibiotic-resistant infections lead to the treatment difficulty, higher costs and increased mortality. In Thailand, infections with drug-resistant bacteria have caused at least 38,000 deaths, incurred US$84.6–202.8 million in direct medical costs and more than US$ 1333 million in indirect costs (Sumpradit et al., 2012).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most widely found pathogens in public healthcare worldwide (Stefani et al., 2012). MRSA causes a range of serious illnesses in the skin, soft tissue, bone and bloodstream, and can lead to sepsis and death. This is due to the effect of β- lactam resistance in *S. aureus* which is mediated by the production of penicillin-binding protein 2a PBP2a or PBP2′ encoded by the *mec*A gene. In order to control MRSA outbreaks, it is desirable to have a rapid and efficient technique to identify hidden reservoirs of MRSA patients and to apply appropriate isolation precautions. Screening method for MRSA is a key success for infection control. The detection of MRSA in clinical specimens by standard clinical microbiology testing such as disk susceptibility tests or broth dilution usually takes one to two days to obtain the result (Cosgrove and Fowler, 2008), so these methods are not appropriate for rapid screening. To accelerate the screening process, molecular techniques such as

* Corresponding author at: Toemsak Srikhirin Physics Department, Faculty of Science, Mahidol University, Rama VI Rd., Phayathai, Bangkok 10400, Thailand.
E-mail address: sctsk@mahidol.ac.th (T. Srikhirin).

http://dx.doi.org/10.1016/j.bios.2015.06.038
0956-5663/

multiplex or real-time fluorescence PCR have been investigated for the direct detection of MRSA in blood cultures in routine laboratories (Grobner and Kempf, 2007; Louie et al., 2002). However, PCR requires comparatively strict template DNA preparation and hours of operating time to have enough end product for detection (Xu et al., 2012). In addition, the sensitivity of PCR can be compromised by PCR inhibitors present in biological samples.

Loop-mediated isothermal amplification (LAMP), as an alternative assay, is a technique for nucleic acid amplification with high specificity, sensitivity and less time consuming. A target nucleotide sequence is amplified on the basis of strand displacement activity by the *Bst* DNA polymerase large fragment (Tomita et al., 2008). A set of four primers, two inner and two outer primers recognizing six different target sequence regions, provides high specificity. The reaction is conducted under isothermal conditions for 60 min in a water bath or a heating block. LAMP assays have been applied for rapid screening tests for several infectious diseases such as malaria and tuberculosis (Aryan et al., 2010; Polley et al., 2013).

Currently, the LAMP reaction can be detected in real time by measuring the turbidity of its DNA polymerization by-product (magnesium pyrophosphate, $Mg_2P_2O_7$). However, turbidity is proportional to the amount of DNA produced and the reaction time. The detection of turbidity changes in a small reaction volume is difficult to observe by the naked eye. In addition, the LAMP technique can also provide turbid samples from non-specific sequences, leading to false positive results. To avoid this drawback, hybridization of the detection probes by a surface sensing method is an alternative for the detection of LAMP amplicons rather than conventional gel electrophoresis, followed by ethidium bromide staining.

Surface plasmon resonance imaging (SPR imaging) measures the refractive index changes at the interface between a metallic layer and a dielectric or sample layer (Puttharugsa et al., 2011). SPR biosensors are capable of detecting interactions between an analyte and an immobilized ligand on the sensor surface in real time without the need to label molecules. This technique has the ability to monitor changes in a large area in real time by monitoring the change in the region of interest (ROI). SPR imaging has been successfully used in medical diagnosis, food industry and in environmental (Piliarik et al., 2009; Shankaran et al., 2007).

In this study, SPR imaging was combined with loop-mediated isothermal amplification to identify *S. aureus* by the detection of *fem*B and to detect antibiotic resistance by the detection of *mec*A in methicillin-resistant *S. aureus* (MRSA) from direct clinical specimens. Samples were verified regarding the species and resistance to methicillin was confirmed by the coagulase and antimicrobial susceptibility tests, respectively, which were used as the gold standard techniques. All of the samples assessed by our detection system were unknown samples. The LAMP assay was performed over the sensor surface to detect MRSA followed by SPR analysis. We have fabricated an in-house biochip array platform for the detection of *fem*B and *mec*A LAMP amplicons. Hybridization was monitored by SPR under a variety of conditions (surface density and buffer concentration) to determine the optimum conditions for sensing. In addition, we also studied SPR sensitivity using two approaches. First, the limit of detection of SPR was validated using 10-fold serial dilutions of LAMP amplicons with both direct detection and signal enhancement with gold nanoparticles to increase SPR sensitivity. Second, the detection limit for the direct detection of various concentrations of *S. aureus* DNA template in LAMP reactions was determined. A combination of SPR–LAMP system was successfully used for the detection of MRSA in clinical specimens without the need for labeling. The identification of *S. aureus* species and resistance detection were performed in array format, taking advantage of the kinetic monitoring of molecular interactions. The sensitivity and specificity were the same as those of the gold standard techniques.

2. Material and methods

2.1. Materials and reagents

A gold sensor chip coated on BK7 glass with 5 nm chromium as an adhesive layer and 50 nm of gold was purchased from Ssens (Netherland). 11-Mercapto-undecanoic acid (11-MUA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (Singapore). Streptavidin (SA) was obtained from AbD Serotec MorphoSys (United Kingdom). Deionized water was obtained from a Millipore unit (Millipore, USA). Primers and biotinylated probes was bought from Bio Basic Inc. (Canada).

2.2. Bacterial strains

Twelve clinically relevant and non-relevant MRSA strains were used to evaluate and optimize the specificity and sensitivity of LAMP assay (Supplementary data Table 1S). To evaluate the SPR–LAMP assay, 90 direct clinical specimens were obtained from positive hemoculture bottles and sputum, including 45 methicillin-resistant *S. aureus* (MRSA), 25 methicillin-susceptible *S. aureus* (MSSA) and 20 methicillin-resistant coagulase negative staphylococcus (MRCNS). All samples were obtained from Clinical Microbiology unit, Ramathibodi Hospital, Bangkok, Thailand.

2.3. DNA extraction

The colonies of MRSA and MSSA were collected and prepared in 10 McFarland standard, which is equal to 1×10^9 copies/ml based on single fragments per cell (Hiramatsu et al., 2001). The sample was diluted with water in a 1000-fold dilution to 1×10^6 copies/ml in a total volume of 1 ml. A simple boiling method was used for DNA extraction by heating at 100 °C for 15 min followed by centrifugation at 12,000*g* for 10 min. The supernatant was transferred to a new tube and the DNA concentration was measured by ND-1000 spectrophotometer analysis (Nanodrop Technologies Inc., USA) at 260 and 280 nm. All direct clinical specimens were processed by PrepMan Extraction (Invitrogen, USA) following the manufacturer's protocol. To determine the detection limit of the LAMP–SPR sensing test, samples were diluted in 10-fold serial dilutions ranging from 10^6 to 1 copies/ml.

2.4. Synthesis of LAMP products

LAMP primers for MRSA detection were designed according to the published nucleotide sequence of *fem*B and *mec*A (The GenBank accession no. BA000017) by Primer Explorer version 3 (Eiken Chemical, Japan). The details of the primers are listed in Table 1. The *fem*B/*mec*A-LAMP assay was carried out in a final volume of 25 μl of a reaction mixture containing: 2 μM each of the inner primers FIP and BIP, 0.2 μM each of the outer primers F3 and B3, 1.4 mM of dNTP mix (Promega, USA), 60 mM betaine (Sigma-Aldrich, USA), 6 mM $MgSO_4$ (Sigma-Aldrich, USA), 8 U of the *Bst* DNA polymerase large fragment (New England Biolabs, USA), 1X of the supplied buffer and 1 μl of template DNA extracted from MRSA and MSSA. The reaction temperature and time were optimized at 63 °C for 60 min to allow for the specific and rapid amplification of MRSA detection. LAMP products were analyzed by 2% agarose gels electrophoresis.

2.5. Preparation of DNA modified gold nanoparticles

A gold nanoparticles suspension was synthesized by the reduction of chloroauric acid ($HAuCl_4$) solution by controlling pH of sodium citrate (Patungwasa and Hodak, 2008) and then

Table 1
Oligonucleotide primers and probes for MRSA detection.

Primers and probe name	Sequences (5′–3′)
***fem*B**	
F3	ATCACATGGTTACGAGCA
B3	TCACGTTCAAGGAATCTGA
FIP	TACCTTCAAGGTTTAATACGCCCAT-**TTTT**-TCATGGCTTTACAACTGAGT
BIP	ACACCCGAAACATTGAAAAAGACA-**TTTT**-CTTTAACACCATAGTTTATCGCTT
***mec*A**	
F3	CCAATTTTGATCCATTTGTTGTT
B3	CATAGCGTCATTATTCCAGG
FIP	ATAGGCATCGTTCCAAAGAATGT-**TTTT**-ACTTAGTTCTTTAGCGATTGC
BIP	GTTTCGGTCTAAAATTTTACCACGT-**TTTT**-AATGCAGAAAGACCAAAGC
*fem*B biotinylated probe	Biotin-T_{10}-TCATGGCTTTACAACTGAGT
*mec*A biotinylated probe	Biotin-T_{10}-ACTTAGTTCTTTAGCGATTGC
Positive control probe	Biotin-T_{10}-TGACGAAAAACCAGAT
*mec*A thiolated probe	SH-T_{10}-TTTATAATCTTTTTTAGAT

characterized by ND-1000 spectrophotometer (Nanodrop Technologies Inc., USA). Thiolated DNA probes conjugated with gold nanoparticle (AuNP) were prepared as previously described (Hill and Mirkin, 2006). First, a freshly purified thiol-modified oligonucleotide probe (5 nmol) was added to 1 ml of the gold nanoparticle solution and left at room temperature for 20 h. Phosphate buffer and SDS were added to the mixture to a final concentration at 10 mM and 0.1% (w/v), respectively. The mixture was shaken gently at room temperature for 30 min before the salting buffer (10 mM phosphate buffer, 2 M NaCl, pH 7.0) was added successively to obtain a final concentration of 0.3 M NaCl and left overnight. Unconjugated oligonucleotide probes were removed by centrifugation at 13,000 rpm for 20 min and washed twice with 0.1 M phosphate buffer saline (PBS, pH 7.4) containing 0.1% SDS. DNA-AuNPs were re-dispersed in the same buffer and kept at 4 °C.

2.6. Sensor surface preparation

SPR gold sensor chips were cleaned with piranha solution (a 70:30 mixture of H_2SO_4:H_2O_2) for 5 min, then rinsed with deionized water and ethanol. The sensor chips were dried in a stream of nitrogen gas (N_2) and treated in a UV-ozone cleaner (Jelight, USA) for 20 min. SPR gold sensor chips were then immersed in 11-mercapto-undecanoic acid (1 mM in ethanol solution) for 18 h. After the reaction, the sensor chips were washed with ethanol and dried in a stream of nitrogen gas.

2.7. Biochip array fabrication

Biotinylated probes were diluted at a concentration of 0.1 μM in PBS-EP buffer (450 mM NaCl, 20 mM Na_2HPO_4, 0.1 mM EDTA and 0.005% Tween 20). Probes with three different sequences were used: *fem*B, *mec*A and the positive control (Table 1). A 40 μg/ml solution of streptavidin was injected on to the sensor surface and incubated for 15 min, followed by ethanolamine pH 8.5 for 10 min to block active sites on the surface. The biotinylated probes were injected into the PDMS flow channels and incubated for 30 min at room temperature as shown in supplementary data Fig. 1S (step A) and 1S (step B). The sensor surface was dried in an air flow and the PDMS flow cell was removed. After that, the sensor chip was rotated 90° and new PDMS flow channels were placed on the sensor chip. Next, PBS-EP buffer was flown through the sensor chip prior to detection.

2.8. Reusability of the sensor surface

The LAMP specific target was removed from the surface probe by 5 mM NaOH. To test the reusability of the sensor surface, 100 ng/μl of LAMP products which were amplified from clinical specimens were passed over the immobilized biotinylated probe at a flow rate of 100 μl/min to remove the bound LAMP amplicons. This was repeated six times and the average specific binding was measured.

2.9. Methicillin-resistant S. aureus detection

LAMP amplicon products of *fem*B and *mec*A were prepared according the method described above. Before SPR analysis, PBS-EP buffer was added to the sample tube at a 1:9 ratio and incubated at 95 °C for 4 min followed by 1 min on ice. The samples were tested by passing over an immobilized biotinylated probe surface in direct and sandwich detection assays as shown in supplementary data Fig. 1S (step C) and (step D). The sandwich assay was only used to evaluate the sensitivity of the SPR set up. Non-specific binding was also observed by cross-reaction with surface attached probes on the biochip array platform. The signal was considered to be specific signals when the value was higher than the average non-specific signal plus three standard deviation ($3 \times SD$). After hybridization, the surface could be regenerated with 5 mM NaOH flushed with buffer, rendering the surface ready for a new hybridization reaction.

3. Results and discussion

3.1. Effect of probe density

The biotinylated single stranded DNA (ssDNA) on the two dimensional SAMs surface was investigated, followed by target hybridization. The immobilization of streptavidin (step A in Fig. 1S) was carried out by varying the streptavidin concentration in the range of 500 μg/ml to 10 μg/ml; the results are shown in Fig. 1 (black squares). Streptavidin was covalently linked to the activated SAMs monolayer via amine linkage through the EDC/NHS reaction (Su et al., 2005a). Immobilization was concentration dependent at a low streptavidin concentration and reached saturation at a streptavidin concentration around 500 μg/ml due to the depletion

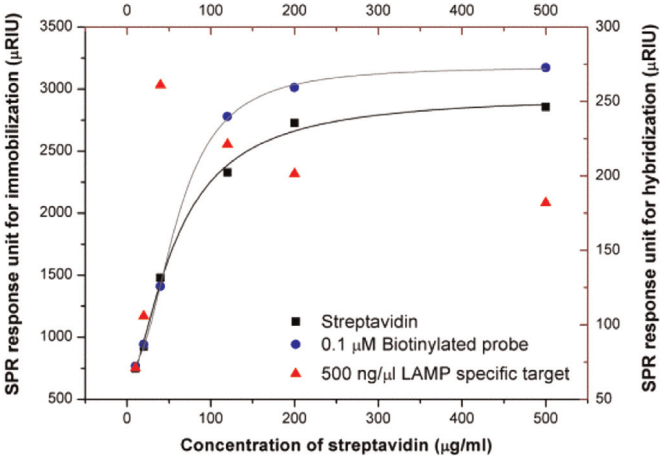

Fig. 1. The effect of the amount of immobilized streptavidin (left axis) as a function of the streptavidin concentration (*x* axis) represented by squares. The amount of the 0.1 μM biotinylated probe that bound to immobilized streptavidin via streptavidin-biotin binding is represented by circles. The triangles represent the degree of hybridization of LAMP specific target MRSA with the immobilized biotinylated probe at various surface concentrations.

of available interacting sites on the SAM surface. The amount of the biotinylated ssDNA probe depends on the available binding site from immobilized streptavidin. As expected, the amount of biotinylated ssDNA bound to the immobilized streptavidin followed the same trend as the streptavidin binding curve (blue circles). The reaction between biotin and streptavidin is a strong interaction with high affinity (Kambhampati and Knoll, 2002) and was found to be advantageous for the surface regeneration step because these molecules did not dissociate by the regenerating solution. It can be seen that the amount of immobilized ssDNA increased and reached a maximum when the concentration of streptavidin was higher than 200 μg/ml. The ssDNA was immobilized and the hybridization process with LAMP amplicons was monitored by SPR. We found that 40 μg/ml of streptavidin on the SAM surface yielded the highest SPR hybridization signal (Fig. 1, right axis).

We assumed that there was an effect of electrostatic repulsion and steric hindrance at surface. At 40 μg/ml of streptavidin, the binding stoichiometry between the biotinylated probe and streptavidin was 0.88:0.51. This means that, on average 1.7 probe strands were immobilized on each streptavidin molecule. The surface concentration for the streptavidin monolayer was 1.96×10^{12} molecules/cm^2 and the area occupied per molecule was 51 nm^2. The area occupied per molecule of each streptavidin was less than 51 nm^2, i.e. 22, 24 and 43 nm^2. We found, on average 2.3 probe strands on each streptavidin molecule and the average surface concentration of the probe was $\sim 2.4 \times 10^{12}$ molecules/cm^2, which is close to the high probe densities defined by Peterson et al. (2002). It is not surprising that the high surface probe concentration gave a low hybridization signal because the combination of electrostatic repulsion and steric hindrance may have prevented the incoming DNA target molecules from diffusing into the probe layer, which has been reported in previous studies (Su et al., 2005b; Wong and Melosh, 2010; Yu et al., 2004). We suggest that the amount of surface-bound DNA probes must be optimized to reduce the steric and electrostatic repulsion effects between neighboring negatively charged probes. Finally, it was determined the immobilization concentration for streptavidin at 40 μg/ml was an appropriate concentration for this system.

3.2. Effect of ionic strength

To investigate whether the ionic strength had an effect on hybridization efficiency, different concentrations of the LAMP specific target and immobilized negatively-charged probes were tested in a series of sodium chloride concentrations ranging of 150–750 mM. The relationship between ionic strength and the hybridization signal was established by plotting the SPR signal versus the sodium concentration (Fig. 2). The hybridization signals were found to increase with sodium concentration from 150 mM to 450 mM, then started to decrease as the sodium concentration increased, similar to a previous study by Yao et al. (2005). A low salt concentrations, the immobilized biotinylated probe may have hindered the access of free LAMP amplicons from the solution to the surface by electrostatic repulsion. The number of ions may have been insufficient to minimize the electrostatic repulsion between surface-tethered probes (Ravan et al., 2014).

As a result, the immobilized surface probe electrostatically repels the free LAMP amplicons in the bulk solution. Therefore, high salts conditions were used to minimize the electrostatic interaction since counterions could neutralize the negatively charged DNA backbone and hence decrease the repulsion of DNA. However, if the system has an excess number of salt ions in the ambient solution, this will lead to two possible outcomes. First, it might interfere with the DNA chain by forming a coiled conformation which can obstruct DNA hybridization (Wang et al., 2013). Second,

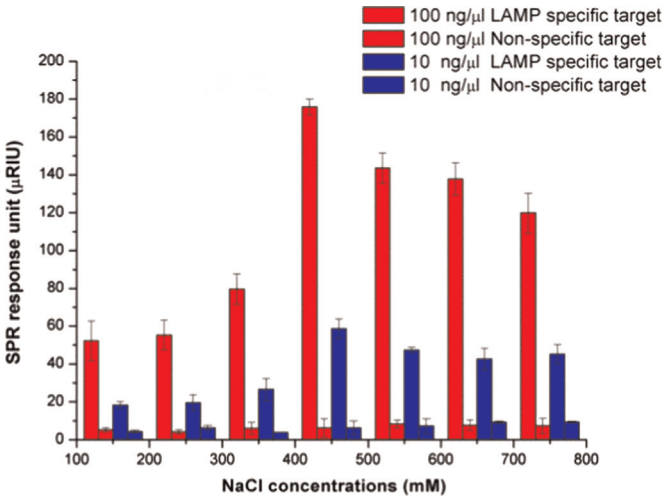

Fig. 2. The interaction of immobilized biotinylated probe and LAMP specific target (MRSA) and non-specific target (MSSA) at different concentrations of sodium chloride (NaCl) in the range of 150–750 mM. The error bars represent the standard deviations.

salt ions can shield the surface probe DNA or free DNA molecules in the bulk solution, which can inhibit the formation of a double helix structure (Springer et al., 2010). As a result, the SPR signal will be decreased. In this work, we observe that the maximum hybridization signal was achieved at 450 mM NaCl.

3.3. Limit of detection (LOD) of the SPR setup

Although this study aimed to screen for the existence of *fem*B and *mec*A LAMP amplicons, we were also interested in validating the LOD of the SPR setup. Thus, a set of 10-fold serial dilutions of LAMP amplicons was tested for binding with the immobilized probe as shown in Supplementary data Fig. 2S(A). The specific interaction of the immobilized biotinylated probe and the 0.23 kbp *mec*A-LAMP product on the streptavidin surface, including DNA-AuNP enhancement, occurred on a log scale. The LOD is calculated from the average specific signal compared with average non-specific signal plus three time standard deviation ($3 \times$ SD). The amount of specific binding was directly related to the LAMP product concentration in the reaction. The LOD in the direct detection assay was 0.1 ng/μl with 39 ± 9 μRIU (black line) and the non-specific signal was 27 μRIU.

To increase the sensitivity of the SPR assay, the signal from the direct detection assay at 10^{-10} to 10 ng/μl was amplified using a sandwich assay. The LOD for the LAMP products increased to 10^{-9} ng/μl with 41 ± 12 μRIU (gray line) as shown in Supplementary data Fig. 2S(A). The results revealed that DNA-AuNPs increased the sensitivity of SPR by increasing the refractive index change generated by the adsorption of DNA molecules at low concentrations. These signals were significantly higher than the non-specific binding signal in which DNA-AuNPs were passed over the immobilized biotinylated probe without MRSA as the negative control channel, yielding a signal of 28 μRIU. The signal amplification to 10^{-9} ng/μl in our study was due to the structure of the large LAMP amplicons which had a repeating unit in the sequence. In Supplementary data Fig. 2S(B), FE-SEM images were used to confirm the presence of amplified AuNPs on the sensor surface. As expected, a cluster of DNA-AuNPs was found on the sensor chip which occurred due to hybridization between DNA-AuNPs and the LAMP amplicons. Non-specific binding from DNA-AuNPs was not observed in negative control samples.

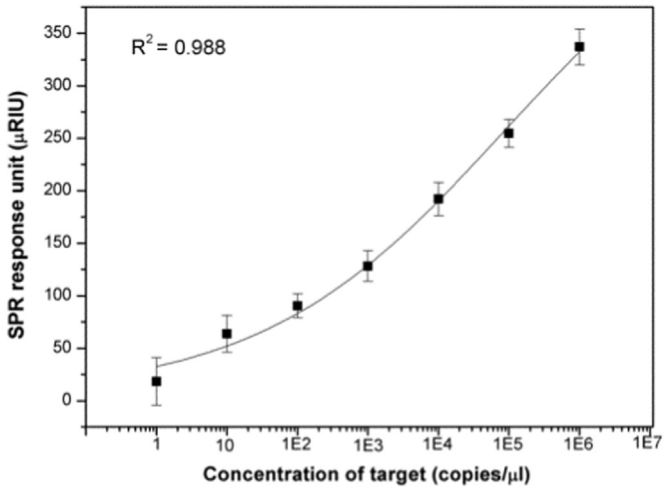

Fig. 3. Calibration curve for the interaction of the immobilized biotinylated probe with LAMP amplifications in different amounts of *S. aureus* DNA template on the streptavidin sensor surface. The limit of detection was 10 copies/μl.

3.4. Detection limit of LAMP–SPR sensing

We set up a model to test the limit of detection for LAMP–SPR sensing by diluting the *S. aureus* template DNA, as described previously. The samples were run through the LAMP assay before detection by SPR. Fig. 3 shows the calibration curve of specific binding which directly related to the amount of *S. aureus* template DNA in the LAMP reactions in the range of $1–1\times10^6$ copies/μl detected by SPR. A sigmoidal curve yielded the best fit for the experimental data with a correlation efficient of 0.988. Establishing the optimized conditions, including the probe density and the salt concentration of the testing solution, was found to be an effective approach for SPR detection of LAMP amplicons from MRSA. The detection limit for LAMP–SPR sensing was 10 copies/μl with 64 ± 14 μRIU which was higher than the average non-specific signal of MSSA plus three standard deviations ($3\times$ SD), 37 μRIU. In addition, there was no need to amplify the SPR signal because the LAMP assay could help to amplify the amount of DNA from low to a tremendous level. Thus, we suggest that LAMP–SPR sensing could be used to detect MRSA at as low as 10 copies/μl in clinical samples.

3.5. Reusability of the sensor surface

The reusability of the sensor surface was tested by inject 5 mM NaOH to remove specifically bound LAMP amplicons from the immobilized biotinylated probe. Fig. 3S in Supplementary data shows six repeated injections of LAMP amplicons in one experiment. We calculated the intra-assay coefficients of variability (CV) to assess the reusability of the sensor surface. The acceptable %CV should be in the range of 5–10% (Wians, 2009). It was found that from Samples no. 1–5 the value of %CV was less than 10%; the %CV increased over 10% for Sample no. 6. The average specific binding signal in the five injections was 131 ± 8 μRIU. The non-specific binding signal was insignificant when the sensor surface was blocked with ethanolamine (pH 8.5) without the application of the biotinylated probe. The results thus confirmed that 5 mM NaOH can be used to regenerate the bound LAMP specific target from the surface without deteriorating the functional streptavidin on the sensor surface. The sensor surface can be reused up to five times.

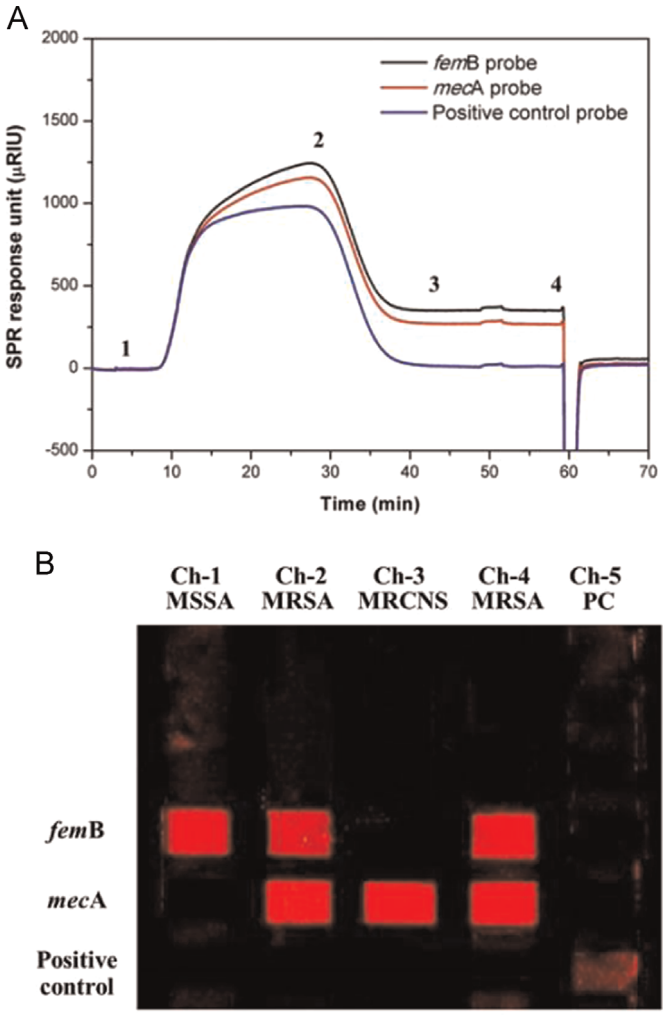

Fig. 4. (A) SPR sensorgram demonstrating the detection of MRSA–LAMP amplicons from clinical specimens on a streptavidin sensor surface which was related to channel 4 (Ch-4) in the SPR image. Point 1, the SPR signal was obtained as baseline. Point 2, the change in the SPR signal reached a plateau and decreased due to unbound LAMP amplicons removed from the streptavidin sensor surface. Point 3, the SPR signal was stable. Point 4, the SPR signal went back to the initial baseline after regeneration of the sensor surface with 5 mM NaOH. (B) SPR image of the DNA array. Each row was immobilized with three different detection biotinylated probed. Each channel was tested with different LAMP amplicons. The positive control (PC) sample is shown in channel 5. A low SPR signal changed is represented by a dark color, and a high SPR signal change is represented by a bright color. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.6. DNA arrays for the detection of MRSA in clinical specimens

A total of 90 staphylococcus isolates from clinical specimens, including 30 sputum and 60 blood hemoculture samples, were tested by LAMP followed by SPR. For analysis, MRSA were designated as positive for *fem*B and *mec*A, but other strains such as MSSA had only *fem*B and MRCNS had only *mec*A, respectively. *fem*B and *mec*A LAMP amplicons bound to the immobilized biotinylated probe were observed as bright spots on the monitor. The brightness of the spots is directly proportion to the SPR signal. This means that a high SPR signal gave a brighter spot, indicating more LAMP specific target bound to the sensor surface. Fig. 4(A) shows the SPR sensorgram for the detection of MRSA in LAMP products that were amplified from the DNA template in direct clinical specimens which was related to the channel 4 array imaging as shown in Fig. 4(B). The image shows flow channel rows immobilized with the biotinylated detection probes in the following

order: *fem*B, *mec*A and the positive control probe, respectively. Each channel from left to right shows bright spots for MSSA, MRSA, MRCNS, MRSA and the positive control, respectively. The area of bright spots in each row and channel were defined as ROIs for measuring the SPR signals. All 90 samples were identified correctly and were consistent with the results from the coagulase and antimicrobial susceptibility tests. The non-specific binding of proteins in the master mix for the LAMP assay did not interfere with the sensor surface. These results suggest that a streptavidin sensor surface with immobilized biotinylated probes in an array platform can be used to detect MRSA–LAMP amplicons in clinical specimens without non-specific binding of proteins in the LAMP master mix. The possibility of combining more than one or two samples in the array format may shorten the analysis time.

4. Conclusion

In this study, we have presented a sensitive and selective DNA sensor for MRSA detection based on LAMP–SPR. The sensor array platform can detect MRSA (*fem*B and *mec*A) LAMP amplicons which were amplified from direct clinical specimens with high specificity and without interference from non-specific adsorbed proteins in the LAMP master mix. The results from our system showed 100% consistency with the coagulase and antimicrobial susceptibility tests. The LAMP–SPR platform provides the possibility of a high throughput technique because it can be applied for multi-target analysis with no labeling required. Future work will focus on DNA arrays with higher numbers of probe spots to detect multiplex drug resistance genes in other bacterial strains. In addition, this sensing system can be readily applied in clinical diagnostic laboratories for the detection of a broad range of pathogens by designing the DNA primer and probe sequences.

Acknowledgment

This work was supported by the National for Electronics and Computer Technology Center (NECTEC), Laboratory of Clinical Microbiology, Department of Pathology, Ramathibodi Hospital and Bioengineering and Sensing Technology Laboratory was partially supported by National Center for Genetic Engineering and Biotechnology (BIOTEC). Financial support for this project from the Center of Excellence (COE) of National Nanotechnology Center (NANOTEC) is gratefully acknowledged.

Appendix A. Suplementary Information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2015.06.038.

References

Aryan, E., Makvandi, M., Farajzadeh, A., Huygen, K., Bifani, P., Mousavi, S.L., Fateh, A., Jelodar, A., Gouya, M.M., Romano, M., 2010. Microbiol. Res. 165 (3), 211–220.
Cosgrove, S.E., Fowler, J., 2008. Clin. Infect. Dis. 46, 386–393.
Grobner, S., Kempf, V.A., 2007. Eur. J. Clin. Microbiol. 26 (10), 751–754.
Hill, H.D., Mirkin, C.A., 2006. Nat. Protoc. 1 (1), 324–336.
Hiramatsu, K., Cui, L., Kuroda, M., Ito, T., 2001. Trends Microbiol. 9 (10), 486–493.
Kambhampati, D., Knoll, W., 2002. Biotin-streptavidin sensor surface: a versatile platform for performing DNA hybridization interactions. In: Mobius, D., Miller, R. (Eds.), Studies in Interface Science. Elsevier Science, Amsterdam, pp. 279–316.
Levy, S.B., Marshall, B., 2004. Nat. Med. 10 (Suppl. 12), S122–S129.
Louie, L., Goodfellow, J., Glatt, P.M., Louie, M., Simor, A.E., 2002. J. Clin. Microbiol. 40 (8), 2786–2790.
Patungwasa, W., Hodak, J.H., 2008. Mater. Chem. Phys. 108 (1), 45–54.
Peterson, A.W., Wolf, L.K., Georgiadis, R.M., 2002. J. Am. Chem. Soc. 124, 14601–14607.
Piliarik, M., Parova, L., Homola, J., 2009. Biosens. Bioelectron. 24 (5), 1399–1404.
Polley, S.D., Gonzalez, I.J., Mohamed, D., Daly, R., Bowers, K., Watson, J., Mewse, E., Armstrong, M., Gray, C., Perkins, M.D., Bell, D., Kanda, H., Tomita, N., Kubota, Y., Mori, Y., Chiodini, P.L., Sutherland, C.J., 2013. J. Infect. Dis. 208 (4), 637–644.
Puttharugsa, C., Wangkam, T., Huangkamhang, N., Gajanandana, O., Himananto, O., Sutapun, B., Amarit, R., Somboonkaew, A., Srikhirin, T., 2011. Biosens. Bioelectron. 26 (5), 2341–2346.
Ravan, H., Kashanian, S., Sanadgol, N., Badoei-Dalfard, A., Karami, Z., 2014. Anal. Biochem. 444, 41–46.
Shankaran, D., Gobi, K., Miura, N., 2007. Sens. Actuators B 121 (1), 158–177.
Springer, T., Sipova, H., Vaisocherova, H., Stepanek, J., Homola, J., 2010. Nucleic Acids Res. 38 (20), 7343–7351.
Stefani, S., Chung, D.R., Lindsay, J.A., Friedrich, A.W., Kearns, A.M., Westh, H., Mackenzie, F.M., 2012. Int. J. Antimicrob. Agents 39 (4), 273–282.
Su, X., Wu, Y.-J., Robelek, R., Knoll, W., 2005a. Langmuir 21 (1), 348–353.
Su, X., Wu, Y.J., Knoll, W., 2005b. Biosens. Bioelectron. 21 (5), 719–726.
Sumpradit, N., Chongtrakul, P., Anuwong, K., Pumtong, S., Kongsomboon, K., Butdeemee, P., Khonglormyati, J., Chomyong, S., Tongyoung, P., Losiriwat, S., Seesuk, P., Suwanwaree, P., Tangcharoensathien, V., 2012. Bull. World Health Organ. 90 (12), 905–913.
Tomita, N., Mori, Y., Kanda, H., Notomi, T., 2008. Nat. Protoc. 3 (5), 877–882.
Wang, M., Tian, Y., Cui, S., Qian, L., 2013. Colloids Surf. A 436, 775–781.
Wians, F.H., 2009. Lab. Med. 40 (2), 105–113.
Wong, I.Y., Melosh, N.A., 2010. Biophys. J. 98 (12), 2954–2963.
Xu, Z., Li, L., Chu, J., Peters, B.M., Harris, M.L., Li, B., Shi, L., Shirtliff, M.E., 2012. Food Res. Int. 47 (2), 166–173.
Yao, D., Kim, J., Yu, F., Nielsen, P.E., Sinner, E.K., Knoll, W., 2005. Biophys. J. 88, 2745–2751.
Yu, F., Yao, D., Knoll, W., 2004. Nucleic Acids Res. 32 (9), e75.