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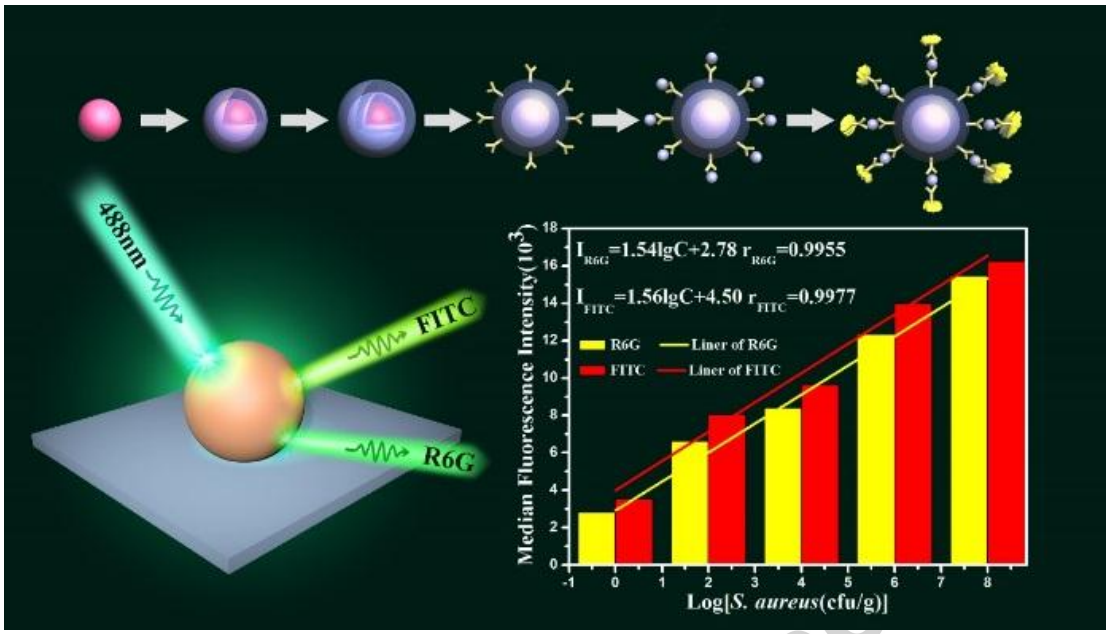
State Key Laboratory of Bioelectronics, School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, P. R. China

Abstract

A novelty bilinear suspension immunoassay biosensor is developed for *Staphylococcus aureus* specific detection. In the present study, a dual-color-based sandwich immunosensor was constructed for sensitive and selective detection of this bacterium. In this bioassay system, the monoclonal antibodies of *Staphylococcus aureus* were immobilized on carboxyl-modified fluorescent microspheres (PSA-R6G) which acted as a capture probe. A secondary fluorescein isothiocyanate (FITC)-labelled antibody of *Staphylococcus aureus* acted as a sensitive reporter antibody. After dual-labelling with R6G and FITC, the enriched *Staphylococcus aureus* bacteria were observed by using the multiparameter flow cytometry analysis. In this method, two regression equations were obtained with I_{FITC}=1.56lgC+4.50 and I_{R6G}=1.54lgC+2.78, respectively. It can be noted that the two slopes were very similar, which indicated the false positives decrease significantly. For general applications, the quantificational measurements of *Staphylococcus aureus* in milk and water samples were also carried out. The suspension immunoassay exhibited an excellent specificity to *Staphylococcus aureus* in contrast to conventional culture-based method.

* State Key Laboratory of Bioelectronics, School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, P. R. China. E-mail: xcxseu@seu.edu.cn or wangmlchem@seu.edu.cn

Graphical abstract



Keywords: *Staphylococcus aureus*; bilinear detection; similar slope; sandwich immunoassay

1. Introduction

The food-borne diseases caused by pathogens have intensive attentions in scientific researches and food industries during the last decades [1]. *Staphylococcus aureus* (*S. aureus*) is one of the main pathogens causing serious food poisoning in humans and animals such as pneumonia, pseudomembranous colitis, pericarditis and even sepsis [2-4]. Usually, the *S. aureus* is found in air, water, dust, soil, human and animal skins. In addition, it can be transmitted not only by direct contact with an infected person or animal but also through air droplets or aerosol including sneezes from infected person [5]. *S. aureus* can easily spread from one species to another, including the spread between humans and animals [6]. Hence, timely and accurate detection of *S. aureus* contamination through the efficient methods is essential to the microbial safety of food.

The reliability of traditional microbiological laboratory procedures and colony counting for *S. aureus* detection is undoubted [7]. Even though, these methods are

sensitive but require several hours or even days which is not suitable to conduct field tests and quickly screen pathogens in emergency situations [8]. For these reasons, many kinds of ultrasensitive detection methods such as enzyme linked immunosorbent assay (ELISA) [9], polymerase chain reaction (PCR) technique [10], electrochemical method [11], nanokeeper [12], piezoelectric sensor [13], fluorescence resonance energy transfer (FRET) [14], colorimetric method [15], chemiluminescent reaction (CL) [16], surface-enhanced raman spectroscopy (SERS) [17] and loop mediated isothermal amplification (LAMP) [18] have been employed in the past decades. Moreover, it is restrained in the use of these approaches because of exorbitant fees and sophisticated procedure.

Biosensor technology is a fast and repeatable method for the detecting bacteria or poisons and other biological molecules by using nucleic-acid sequences, aptamers recognition and antibody-antigen recognition. Numerous biosensing approaches have been applied for the detection of *S. aureus* including enzyme-linked immunosorbent assay [9], surface plasmon resonance biosensors [19], atomic force microscopy measurement [20] and fluorescence based immunoassay [21] because of the advantages of antigen-antibody recognition. However, some of the reports have shown the limitations in false positive accuracy which will restrict the widespread utilization. In this regards, further efforts are urgently required for the development of more approaches with cost-effective, simple operation as well as high sensitivity and accuracy.

In recent years, some progress has been made in bacterial detection by the adoption of PS fluorescence microspheres as concentration carriers [22, 23]. For the aim of separation and high sensitivity detection of target pathogens, the fluorescence microspheres have been modified by some biometric identification molecular. In addition, the suspension arrays using encoded micro carriers were a powerful platform for multiplex detection [24]. Hence, this method has been introduced as support carriers using encoding microspheres with one or more fluorescent dyes. Each bead can be bound to different probe molecules which can allow the simultaneous measurements of different biomolecular interactions [25]. Recently, such attempt has

been reported by Deng et al. [26] and they employed carbon dots-encapsulated breakable organosilica nanocapsules (CDs@BONs) as single label for fluorescent immunoassay detection of *S. aureus*. However, the CDs@BONs are even inferior to organic fluorophores because of their significantly higher false positives according to the report. Reasons for the occurrences of false positives are: (1) a high background noise which arose from aggregates and (2) a high level of nonspecific binding in the samples. Therefore, the development of new method to overcome false positives is very urgent. Bilinear detection using two regression equation can decrease the false positives through increasing the similarity of slopes.

Inspired by the mentioned points above, a new method has been proposed and demonstrated here for a sensitive, specific, high accuracy flow cytometry detection of *S. aureus* through combination of two recognition molecules of R6G and FITC. On the basis of this strategy, the *S. aureus* in a mixture containing other nontarget bacteria has been quantified by using antibody-coated PSA-R6G microspheres with fluorescein isothiocyanate (FITC) labelled antibody of *S. aureus*. The *S. aureus* in authentic milk and water samples has the detection recoveries ranging from 97.6% to 102.2%. The R6G and FITC based dual recognition strategy with enriched fluorescence detection has potential applications in screening of food-borne pathogens and clinical diagnosis of bacterial infection.

2. Experimental section

2.1. Reagents and solutions

All of the reagents and materials used in these experiments are without any further purification processes. The styrene, polyvinyl-pyrrolidone (PVP, Mw=55000 g mol^{-1}), polyethylene imine (PEI) solution Triton X-305 and polyacrylic acid (PAA) solution were purchased from Aladdin (www.aladdin-e.com/zh_cn). 2, 2'-Azobis (2-methyl-butyronitrile) (AMBN) was purchased from Wako Pure Chemical Industries Ltd (http://www.wako-chem.co.jp). The N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were purchased from Sigma Aldrich (www.sigmaaldrich.com). The streptavidin and FITC conjugated were purchased from Thermo Fisher Scientific Inc

(www.thermofisher.com). A biotin-labelled goat anti-mouse IgG (H + L) was obtained from Beyotime Biotechnology (www.beyotime.com). A mouse anti-staphylococcus aureus monoclonal antibody was purchased from Abcam (www.abcam.com). The mouse anti-*S. aureus* polyclonal antibodies (FITC) were purchased from Santa Cruz Biotechnology Inc (www.scbt.com).

2.2. Apparatus

The morphology of as-synthesized products was characterized by using a field emission scanning electron microscope (FESEM, Carl Zeiss Ultra Plus, www.zeiss.com). The UV-visible spectra were measured by using a UV-visible spectrophotometer (UV-2600, Shimadzu, www.shimadzu.com). The PL spectra were recorded by using a fluorescence spectrophotometer (F-4600, Hitachi, www.hitachi.co.jp) with Xe lamp as an excitation source. A Malvern Zetasizer Nano ZS was used to measure the zeta potential (www.malvern.com). A flow cytometry analysis was performed by using a Novocyte benchtop flow cytometer (NovoCyte 2060, ACEA Biosciences, www.aceabio.com). The Fourier transform infrared (FTIR) spectra (KBr pellets) were measured using Nicolet 5700 FTIR spectrometer (Nicolet 5700, Thermo Nicolet, www.thermofisher.com).

2.3. *S. aureus* culture, pretreatment and counting

Under the condition of oxygen, *S. aureus* was cultured shakily for 24 h in Lysogeny broth (LB) at 37 °C. Then the *S. aureus* in LB with length of 2 mL were centrifuged at 10000 rpm for 15 min to remove the culture medium and then washed with PBS buffer thrice through centrifugation at 10000 rpm for 15 min. After that, the bacterial suspension was resuspended in PBS buffer and diluted. The cellular concentration was analyzed by flow cytometry via a flow cytometer. The result showed that the concentration of *S. aureus* was about 1×10^{8} cfu g^{-1}. Other bacteria including *Escherichia coli O157:H7*, *Listeria monocytogene*, *Vibrio Parahemolyticus*, *Salmonella*, *Shigella Bogdii* and *Campylobacter* were also cultured shakily for 24 h in Lysogeny broth at 37 °C. The concentrations of bacteria were determined by flow cytometry.

2.4. Synthesis of Rhodamine 6G embedded polystyrene microspheres

A simple synthesis method for the preparation of PS-R6G powder has been proposed in previous literature [27, 28]. During the experimental processes, we used PVP as stabilizer, Triton X-305 as co-stabilizer and AMBN as initiator, styrene as monomer and ethanol as solvent. In 250 mL four-neck flask with condenser and gas inlet, the mixture was stirred until well blended at room temperature. After that, it was deoxygenated for 30 min under nitrogen condition. Then, reaction occurs under oil bath at 70 °C and stirred at 120 rpm. On the other hand, R6G and styrene were dissolved in ethanol. After an hour of copolymerization reaction, the R6G and styrene mixture was added slowly and evenly to the reaction flask within 6 hours. Then, the copolymerization reaction lasted for 24 h. The PS-R6G microspheres were obtained ultimately by centrifugation and vacuum dying.

2.5. Layer-by-layer (LBL) assembly of carboxyl-modified PS-R6G microspheres

In a typical experimental process, the PS-R6G microspheres with homogeneous distribution were made by ultrasonic dispersing technique. Afterwards, the PS-R6G suspension was sequentially assembled by coating of positive PEI and negative PAA to obtain PSA-R6G microspheres. The assembly processes are as shown below: Firstly, positive PEI was added into PS-R6G suspension and agitated magnetically for 30 min. After that, the compound was centrifuged and washed with H_2O for purpose of removing the excess PEI solution. PS-R6G@PEI suspension was obtained by dispersing the final centrifuged deposits into H_2O. Secondly, negative PAA solution was added into PS-R6G@PEI suspension and agitated magnetically for 30 min. Then, the compound was also centrifuged and washed with H_2O for purpose of removing the excess PAA solution. PSA-R6G suspension was obtained by dispersing the final centrifuged deposits into H_2O. The introduction of additional double layers provided carboxyl-modified surfaces of microspheres which accumulate the further links of anti-*S. aureus* mAb for the specificity detection of *S. aureus*. The obtained carboxyl modified PS-R6G fluorescent microspheres were stored at 4 °C.

2.6. Bioconjugation of anti-*S. aureus* mAb and PSA-R6G microspheres

As shown in Fig. 4, the anti-*S. aureus* mAbs can be linked to the surfaces of PSA-R6G microspheres by covalent binding. The covalent binding was occurred

between the carboxyl of PSA-R6G microspheres and the amino of anti-*S. aureus* mAbs through carbodiimide reaction in PBS solution. Initially, the activation of the carboxyl on the surfaces of PSA-R6G microspheres lasted for 20 min in the mixture of 16 μL of monobasic sodium phosphate (0.1 M), 10 μL of EDC and 10 μL of NHS. Then, the activated PSA-R6G microspheres were washed thrice with PBS solution and acquired by using a centrifugation. Next, the obtained microspheres reacted with anti-*S. aureus* mAbs in PBS solution at 37 °C for 2 h. After centrifuging, the anti-*S. aureus* mAb-conjugated PSA-R6G microspheres were washed thrice with PBST for the purpose of removing the excess anti-*S. aureus* mAbs.

2.7. Detection of surface functionality of PSA-R6G microspheres

In order to bio-coupling the anti-*S. aureus* mAbs and PSA-R6G microspheres successfully and satisfactorily, the surface functionalization of PSA-R6G microspheres should be detected through a flow cytometry analysis. The as-prepared microspheres can be labeled with biotin-labeled goat anti-mouse IgG and streptavidin-FITC through specific molecular binding. After that, the results were obtained as median fluorescence intensity (MFI) value through flow cytometry analysis. Concrete operations as follows: the previously acquired anti-*S. aureus* mAb and PSA-R6G microspheres biological coupling structure were collected and incubated with biotin-labeled goat anti-mouse IgG at 37 °C for 1 h. After the above microspheres were washed with PBST, cultured it with streptavidin-FITC further at 37 °C for 30 min. At last, the final labelled microspheres were washed with PBST and suspended in PBS and then analyzed the final labelled microspheres through flow cytometry analysis.

3. Results and discussion

3.1. Morphology and structural characterization

The smooth facets of highly monodispersed PS-R6G microspheres with diameter of 5 μm are depicted in Fig. 1(a) which acts as a flow cytometer substrate. In order to bio-conjugate with biomolecules, the carboxyl-modified fluorescent microspheres of PS-R6G@PEI/PAA (shortened as PSA-R6G) have been prepared through an electrostatic self-assembly method as shown in Fig. 1(b). In the circumstances, the

PS-R6G microspheres can be used for a substrate which coated with positive and negative functional layers of PEI and PAA, respectively. From Fig. 1(b), it can also be observed that the rough external surfaces of microspheres with presence of carboxyl groups. Interestingly, the carboxyl groups greatly promote the interactions with plentiful amino groups with monoclonal antibody as illustrated in Fig. 1(c).

Fig. 1

3.2. Optical properties of PS-R6G microspheres

To explore the luminescence properties of PS-R6G microspheres at room temperature, the PL spectra have been performed as shown in Fig. 2. Interestingly, the PS-R6G microspheres exhibit good luminescence properties. The PL spectrum of PS-R6G exhibits a strong response at the wavelength of 555 nm which confirms the successful decoration of R6G on PS microspheres. When the target bacterium was added, the Anti/S. aureus/Anti sandwich immunosensor was formed. At that condition, the reporter molecules of R6G and FITC were assembled successfully. From Fig. 2, it can also be observed that a PSA-R6G-FITC spectrum exhibits the strong responses at the wavelengths of 523 nm and 555 nm for FITC and R6G, respectively.

Fig. 2

3.3. Biofunctionalization of PSA-R6G microspheres and anti-*S. aureus* mAbs

During the preparations of final products of PSA-R6G as well as intermediate PS-R6G@PEI, the different electric layers have been introduced. So the entire process was controlled by using zeta potentiometer because of potential changes occurred on the surfaces of microspheres and the results are illustrated in Fig. 3. Here, the outermost layer of PSA-R6G comprises with plentiful carboxyl groups which are propitious to the connection with biomolecules. Due to the introduction of the anti-*S. aureus* mAbs as specific recognition biomolecules into PSA-R6G microspheres based on two-step carbodiimide reaction route. Therefore, the anti-*S. aureus* mAbs bio-coupled PSA-R6G microspheres is suitable for the detection of *S. aureus* immunoassay as a novel kind of fluorescent probes.

Fig. 3

Fig. 4

To achieve a better bioconjugation, the concentration of anti-*S. aureus* mAbs have been optimized. The different volume of anti-*S. aureus* mAbs (0, 10, 30, 50, 60, 65, 70, 75, 80, 90. 100, 120 and 140 μg) have been tested for conjugation with PSA-R6G microspheres and fluorescein isothiocyanate (FITC) labelled antibody of *S. aureus*. The different anti-*S. aureus* mAb-conjugated PSA-R6G-FITC microspheres have been collected, and their corresponding MFI values are obtained through flow cytometric analysis. In anti-*S. aureus* mAb-conjugated PSA-R6G microspheres, the best coupling is achieved on account of the highest detection of MFI. The MFI values are changed with respect to addition of different volume of mAbs in the coupling assay as shown in Fig. 5. From the figure, it can also be noted that the MFI values are increased with increase in coupling of mAbs on the surfaces of microspheres between 0 – 80 μg. As shown the inset in Fig. 5, there is a slight increase between 0 - 60 μg. Then a significantly increase appeared between 60 – 80 μg. Moreover, the maximum MFI value is achieved for the coupling of 80 μg of mAbs with PSA-R6G-FITC microspheres. The antigen and antibody reactions generally follow a certain quantity relationship. According to that, the concentration ratio clearly yields a visible response and proportional to antigen antibody fairly or slightly surplus situation of antigen, maximum radical reaction and maximum formation of immune complex deposits. When increasing a ratio of antigen-antibody above 80 μg both reaction speed and amount of sediment decreased rapidly or absence of antigen antibody response. Therefore, the PSA-R6G-FITC microspheres with 80 μg of mAbs are employed in the following bilinear *S. aureus* detection based on suspension immunoassay.

Fig. 5

3.4. FTIR analysis

The FTIR spectra of PS-R6G, PSA-R6G and PSA-R6G-Ab complexes are shown in Fig. 6. O–H stretching vibration at 3400 cm^{-1}, C=O stretching at 1710 cm^{-1} and isopropyl absorption peak at 1384cm^{-1} can be found in the IR spectrum of PSA-R6G. The three new peaks demonstrated the presence of carboxylic acid group. In terms of PS-R6G-Ab analysis, the peak at 3409cm^{-1} belonged to O–H and N–H

stretching vibration. The characteristic absorption band of the peptide bond functional group (–NH-CO-) can be seen at 1550 cm^{-1}. The peak at 1641 cm^{-1} corresponds to the carbonyl group(C=O stretch) of peptide bond and 1267 cm^{-1} denotes the C-N stretch. The peak at 1384cm^{-1} corresponds to isopropyl absorption of polyacrylic acid. These peaks demonstrate the covalent binding in PSA-R6G-Ab complexes.

Fig. 6

3.5. Bilinear *S. aureus* detection based on suspension immunoassay

A detection of *S. aureus* mainly based on specific antibody-antigen recognition between *S. aureus* and anti-*S. aureus* mAb biological coupled PSA-R6G microspheres. The binding abilities for *S. aureus* with anti-*S. aureus* mAbs bioconjugated PSA-R6G microspheres have been experimented with the Anti/S. aureus/Anti sandwich immunosensor as shown in Scheme 1. In addition, the formations of immune complexes usually included the antibody-antigen recognition between anti-*S. aureus* mAbs biological coupled PSA-R6G microspheres and *S. aureus*, then the mouse anti-*S. aureus* polyclonal antibodies (FITC) was biological coupled with the as-prepared fluorescent microspheres according to some of the testing systems.

Scheme 1

To achieve the bilinear detection of *S. aureus* based PSA-R6G microspheres suspension immunoassay, the fluorescent microspheres were incubated in bacteria samples with presence and absence of *S. aureus* (n = 5) for 1 h at 37 °C. After that, the samples were washed PBST and then incubated with 80 μL of mouse anti-*S. aureus* polyclonal for 1 h at 37 °C. Then the final product was washed with PBST and dispersed in PBS. After that, the immunodiagnostic complexes were analyzed with both fluorescence of FITC and R6G.

In order to fulfill the precise immune analysis of *S. aureus* detection, the suspension immune analysis system was designed and the detection of *S. aureus* was carried out in actual water samples. We further analyzed the water samples of S. aureus with different concentrations from 1 cfu g^{-1} to 1×10^{8} cfu g^{-1} which are preliminary treated for 1 h under identical experimental conditions. The results can be showed that the fluorescence intensity of FITC and R6G the anti-*S. aureus* mAbs

bioconjugated PSA-R6G microspheres with *S. aureus* complex were different, which is allowed to distinguish between the presence and absence of *S. aureus*. The MFI values of FITC and R6G are gradually increased when increasing the concentration of *S. aureus* from 1 cfu g^{-1} to 1×10^{8} cfu g^{-1} as shown in Fig. 7. The obtained regression equation for FITC is I_{FITC}=1.56lgC+4.50 with correlation coefficient of 0.9977 and limit of detection (LOD) of 1.15×10^{-1} cfu g^{-1}. Moreover, the yielded regression equation for R6G is I_{R6G}=1.54lgC+2.78 with correlation coefficient of 0.9955 and LOD of 1.12×10^{-1} cfu g^{-1}. It can also be noted that the slopes of two signal molecules as 1.56 and 1.54 for FITC and R6G, respectively. Due to the actions of both signal molecules as reporter molecules in *S. aureus* detection, the similar slope indicates a positive assay of dual-color-based sandwich immunosensor. Here, two factors are critical namely target and ideal signal amplification with minimal false positive signal. According to the report of Kemin Wang et al., the dual-color FCM for pathogen detection is sensitive with dramatic decrement in false positive signals [29, 30]. But there is only one regression equation in these methods. In the present work, the protocol obtained highly efficient PS microspheres for highly sensitive FCM detection and achieved an outstanding accuracy in the detection of *S. aureus*.

Fig. 7

To examine the reactions of anti-*S. aureus* mAbs bioconjugated PSA-R6G microspheres with other pathogens, more than five species of bacteria identified by culture method were also applied in the bilinear detection. In detecting the cross-reactivity between antigen and antibody reaction, the heat-inactivated bacteria, for instance, *Escherichia coli O157:H7*, *Listeria monocytogene*, *Vibrio Parahemolyticus*, *Salmonella*, *Shigella Bogdii* and *Campylobacter* were tested. To test repeatability, three different samples of *S. aureus* were examined five times in triplicate and each experiment was tested back and forth five times. The results can be showed that an accurate identification of *S. aureus* by anti-*S. aureus* mAbs bioconjugated PSA-R6G microspheres with a very high MFI value which confirms an excellent specificity of anti-*S. aureus* mAbs bioconjugated PSA-R6G microspheres to *S. aureus* with no cross reaction with other bacterias as depicted in Fig. 8.

Fig. 8

In order to assess the reliability of this method for the detection of actual samples, tap water, boiled water and milk were selected as actual samples to analyze *S. aureus* quantitatively. The samples were tested after sterilization and the recovery test was tested by adding standard solutions with different concentrations of *S. aureus*. Because of the potential of the assay can be evaluated through the recovery tests. The recovery results labelled by FITC are listed in Table 1. From the table, it can be identified that the acceptable recoveries are presented in the ranging between 98.7% and 101.3% with RSD values of below 4.2%. For R6G, the acceptable recoveries are located in the ranging from 97.6% to 102.2% with RSD values of below 4.9% as listed in Table 2. The results can be proposed that the repeatability of the bilinear detection method based PSA-R6G microspheres suspension immunoassay is very high.

According to above experimental results, the bilinear detection method based PSA-R6G microspheres suspension immunoassay has similar accuracy of with culture method. However, it needed 18 to 96 h to complete the experiment using the culture method [31] while the bilinear detection method based PSA-R6G microspheres suspension immunoassay was completed within 2 h. Hence, the anti-*S. aureus* mAbs bioconjugated PSA-R6G microspheres represent an efficient approach for the detection of *S. aureus* in food substances.

Table. 1

Table. 2

4. Conclusion

A new approach was proposed for the detection of *S. aureus* by using a bilinear suspension immunoassay with combination of bioconjugated fluorescent microspheres and FITC labelled antibody of *S. aureus*. Compared with FCM based single-label luminescent microspheres, the bilinear method dramatically decremented the false positive signals through the similar slopes of the two regression equations. Some of the microspheres possessed a high sensitivity during the detection of Ag in pre-enriched *S. aureus*-contaminated milk and water samples. The lowest LOD of *S.*

aureus content are 1.12×10^{-1} cfu g^{-1} and 1.15×10^{-1} cfu g^{-1} for the signal molecules of R6G and FITC, respectively. Additionally, the suspension immunoassay using PSA-R6G microspheres exhibited a superior accuracy to *S. aureus* with good repeatability and high efficiency in contrast to conventional culture-based method. This work can pave a new strategy to fabricate the luminescent microspheres for the developments of immunoassay techniques.

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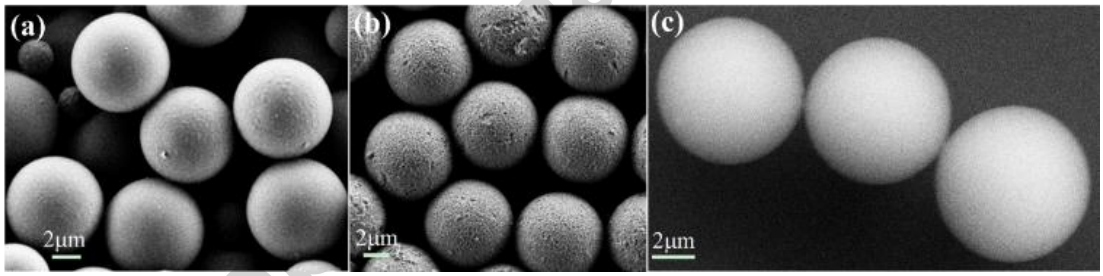


Fig. 1 The SEM image of (a) PS-R6G microspheres, (b) PSA-R6G microspheres prepared by using a LBL electrostatic self-assembly technique and (c) Self-assembly of PSA-R6G microspheres after adding antibody.

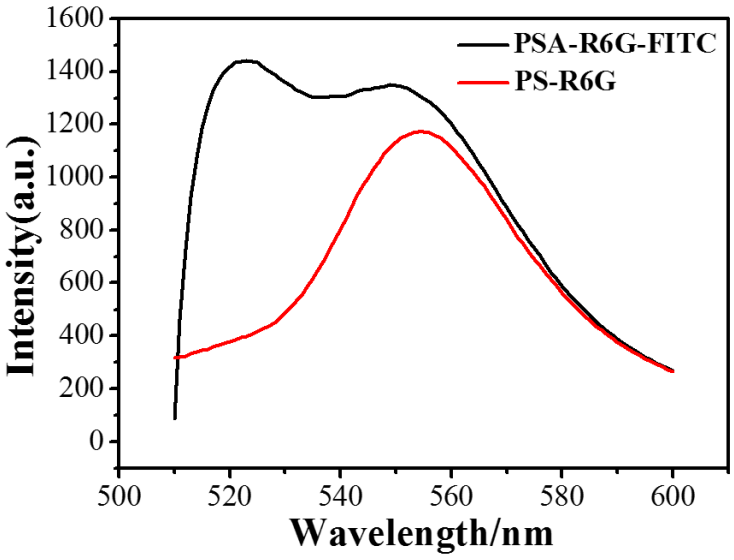


Fig. 2 The PL spectra for PS-R6G matrix and PSA-R6G-FITC matrix

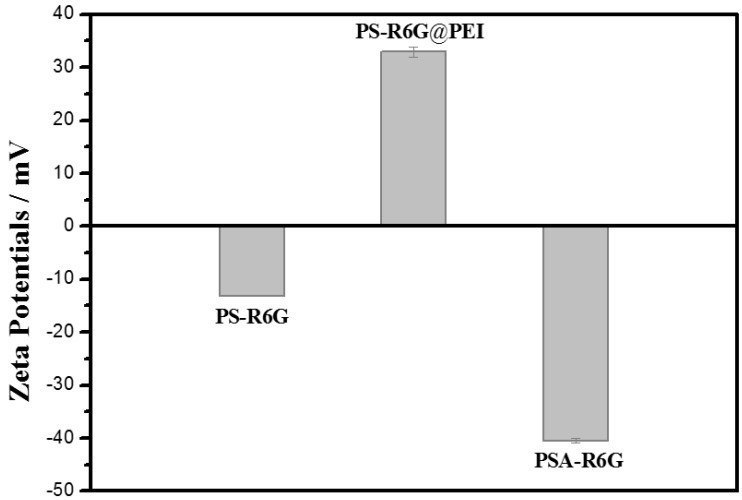


Fig. 3 Zeta potential measurements during the preparation of PSA-R6G microspheres

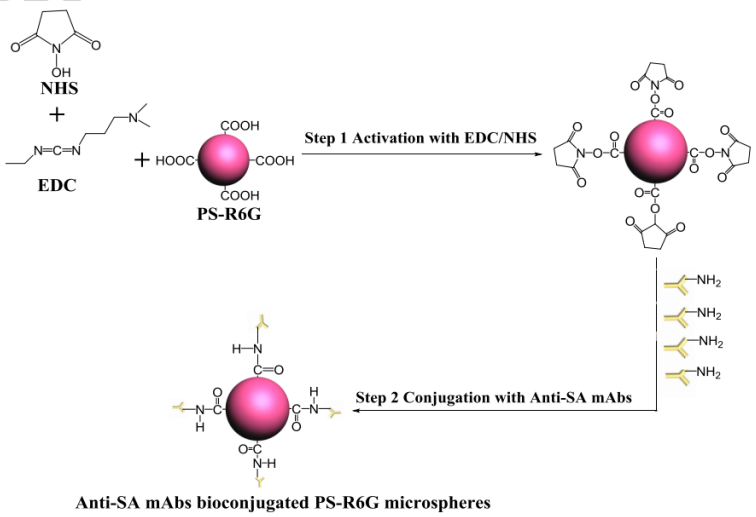


Fig. 4 A schematic view of biofuctionalization steps of PSA-R6G microspheres which conjugated with anti-*S. aureus* mAbs

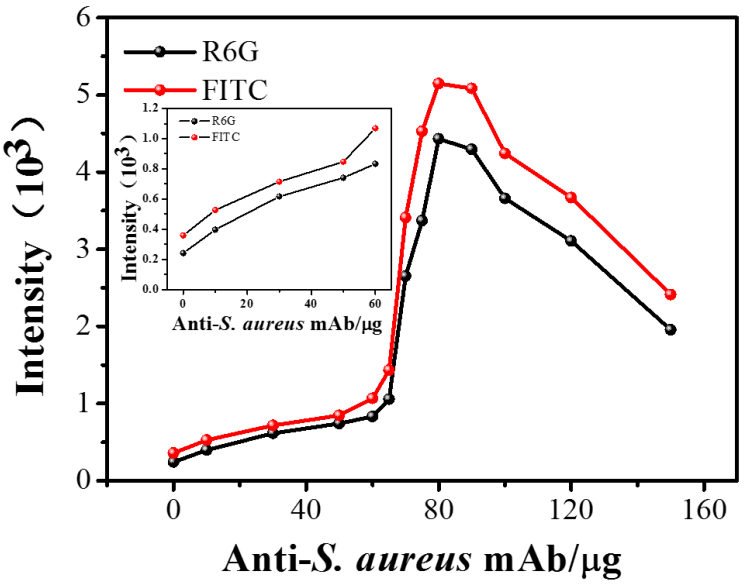


Fig. 5 The variations in MFI values with different amounts of anti-S.aureus mAbs in the binding assay. The inset show the enlarged between 0 and 60 μg.

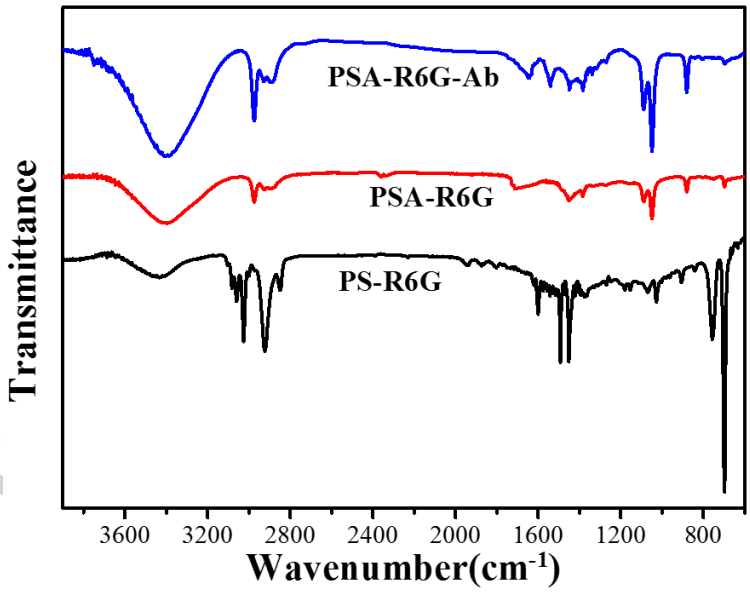
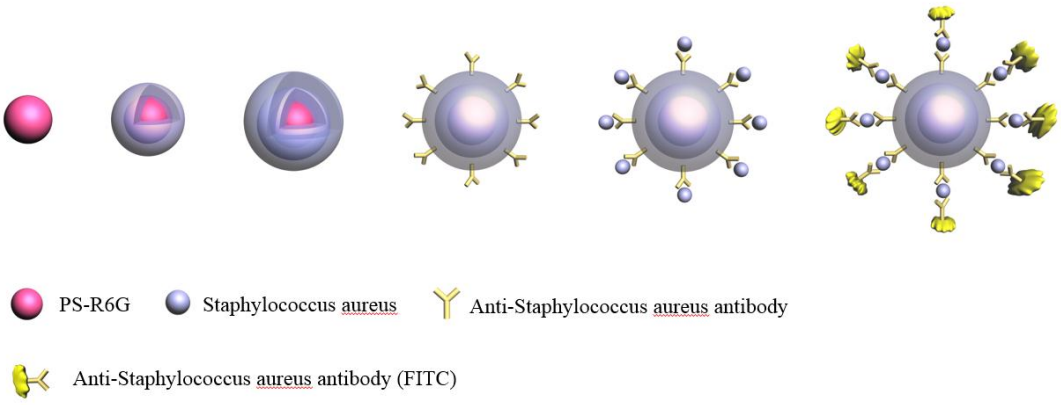


Fig.6 Fourier transform infrared spectrogram of PS-R6G, PSA-R6G and PSA-R6G-Ab complexes.



Scheme 1. A schematic illustration for a formation of immune complex

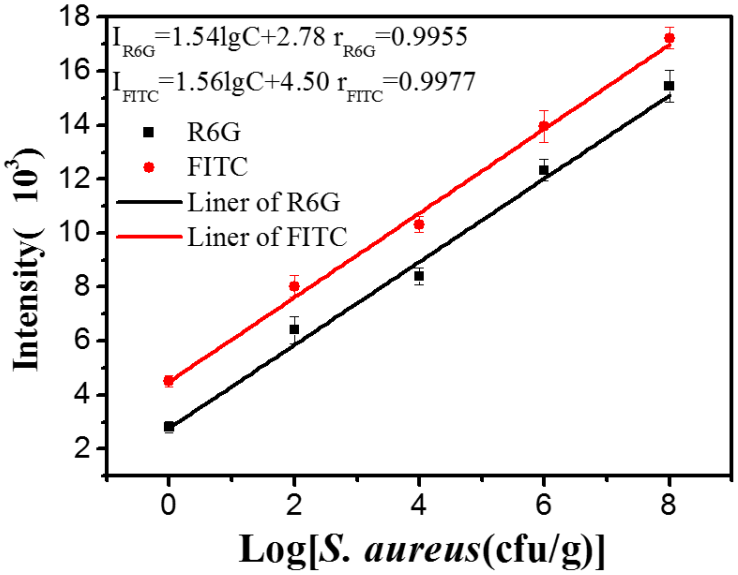


Fig. 7 A plot of MFI and different concentrations of *Staphylococcus aureus* which shows the sensitivity of PSA-R6G microsphere based bilinear detection

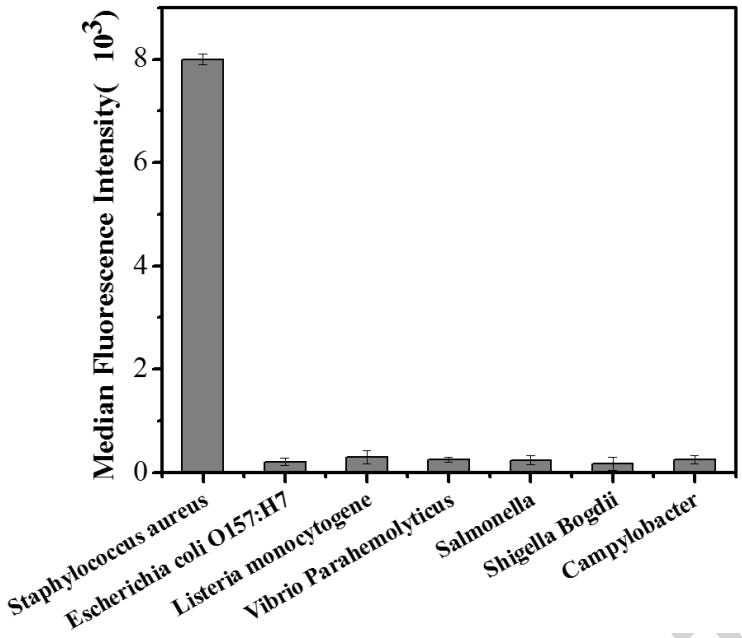


Fig. 8 Specificity of bilinear detection based on PSA-R6G microspheres suspension immunoassay

Table. 1 The bilinear detection method based PSA-R6G microspheres suspension immunoassay which labelled by FITC (n=5) for detecting *S. aureus* from water samples

Sample	**Found** (cfu g^{-1})	**Added** (cfu g^{-1})	**Total found** (cfu g^{-1})	**RSD (%)**	**Recovery (%)**
Tap water	0.99	200	198.4	4.2	98.7%
Boiled water	0.58	200	203.2	3.0	101.3%
Milk	0.07	200	201.1	2.0	100.5%

Table. 2 The bilinear detection method based PSA-R6G microspheres suspension immunoassay which labelled by R6G (n=5) for detecting *S. aureus* from water samples

Sample	**Found** (cfu g^{-1})	**Added** (cfu g^{-1})	**Total found** (cfu g^{-1})	**RSD (%)**	**Recovery (%)**
Tap water	0.99	200	205.4	4.9	102.2%
Boiled water	0.58	200	195.8	2.4	97.6%
Milk	0.07	200	199.1	1.4	99.5%

Highlights

1. A novelty bilinear suspension immunoassay biosensor was developed for *Staphylococcus aureus* specific detection.
2. The slopes of two regression equations are very similar, which indicates the false positives decrease significantly.
3. Through the detection of real samples, it provides an excellent prospect for food safety.