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A microfluidic biosensor for online and sensitive detection of *Salmonella* typhimurium using fluorescence labeling and smartphone video processing

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ABSTRACT

Early screening of foodborne pathogens is a key to ensure food safety. In this study, we developed a microfluidic biosensor for online and sensitive detection of *Salmonella* based on immunomagnetic separation, fluorescence labeling and smartphone video processing. First, the immune magnetic nanoparticles were used to specifically separate and efficiently concentrate the target bacteria and the magnetic bacteria were formed. Then, the magnetic bacteria were labeled with the immune fluorescent microspheres and the fluorescent bacteria were formed. Finally, the fluorescent bacteria were continuously injected into the microfluidic chip on the smartphone-based fluorescent microscopic system, and the fluorescent spots were online counted using the smartphone App based on inter-frame difference algorithm to obtain the amount of the target bacteria. Under the optimal conditions, this proposed biosensor was able to quantitatively detect *Salmonella* typhimurium ranging from 1.4×10^2 to 1.4×10^6 CFU/mL, and its lower detection limit was 58 CFU/mL. This biosensor could be extended for detection of multiple foodborne pathogens using different fluorescent materials.

1. Introduction

Foodborne pathogens are a main factor for the outbreaks of foodborne diseases. According to the World Health Organization (WHO), there are over 150 million diarrheal cases in the world every year, 70% of which are related to foodborne pathogens (World Health Organization, 2018). Among these foodborne pathogens, Salmonella with 2,523 serotypes receives the most concerns (Malorny et al., 2009) since it is widely found in various foods and can result in severe diseases sometimes. At present, there are some existing methods for detection of *Salmonella*. The traditional culture plating is the gold standard method based on bacteria growing in agar-based growth medium. It has high accuracy and sensitivity, however it takes a few days (2–4 d) to obtain final results and is not suitable for in-field detection (Lee et al., 2015; Rohde et al., 2017). Enzyme-linked immunosorbent assay (ELISA) is a recommended method based on the immunological reaction between antigen and antibody. It is rapid (2–4 h), high-throughput and specific, however it has a low sensitivity and is often subject to cross contamination (Miller et al., 2011; Zhang et al., 2017). Polymerase chain reaction (PCR) is recommended for rapid detection of *Salmonella* and featured with short time (2–4 h), high throughput and high sensitivity, but it requires complex DNA extraction procedure and professional facilities (Jyoti et al., 2010; Lungu et al., 2012). Thus, there is an urgent demand on rapid and sensitive detection methods of foodborne pathogens.

As an alternative for bacteria detection, fluorescent biosensors have been often reported to detect *Salmonella* in recent years. In most studies, fluorescent biosensors were developed first using immune magnetic nanoparticles to capture the target bacteria to form the magnetic bacteria, then using fluorescent materials such as fluorescein (Lee et al., 2017) and quantum dots (Yang and Li, 2006) to conjugate the magnetic bacteria to form the fluorescent bacteria, and finally measuring the fluorescent signals to determine the amount of the target bacteria. The reported fluorescent biosensors were often able to detect foodborne bacteria as low as 10^1–10^3 CFU/mL (Bayramoglu et al., 2018; Li et al., 2018). Recently, an interesting fluorescent biosensor was reported by Wang et al. (Wang and Kang, 2016) for bacteria detection using single-stranded DNA aptamers conjugated silica fluorescent nanoparticles to label the target *Salmonella* cells, which then could be counted on a fluorescent microscope to obtain quantitative results. Although

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immunofluorescent assays have been widely used for bacteria detection due to their high sensitivity, good specificity and fast speed (Duedu and French, 2017), they are still faced with some challenges, such as non-specific adsorption to proteins, cells, nucleic acids and other substances in real samples, resulting in inevitable background noises and false positives (Lee et al., 2014; Wang and Duncan, 2017). Thus, combination of immunomagnetic separation and immunofluorescent assays might be promising to develop new rapid, sensitive and non-contact methods for bacteria detection.

In recent years, microfluidics has received extensive attentions and been frequently used for biological detection due to its outstanding advantages, such as small size, less reagent consumption, high reaction efficiency, low cost, easy integration, automatic operation and point-of-care diagnosis (Fang et al., 2010; Kulinsky et al., 2013; Whitesides, 2013). At present, optical detection and electrochemical detection are often used with microfluidics to develop microfluidic biosensors (Hassan et al., 2016; Martinez et al., 2008; Nie et al., 2010; Stott et al., 2010). An example is a microfluidic chip for ultrasensitive detection of *Salmonella* Typhimurium reported by He et al., (2013) using positive dielectrophoresis enrichment and fluorescent nanoparticles label. It could detect *Salmonella* as low as 56 CFU/mL in 2 h. Another interesting sample is a microfluidic chip with magnetically fluidized bed reported by Srbova et al., (2017) for immune separation of *Salmonella* in milk. This microfluidic chip had a separation efficiency of up to 99% for *Salmonella*. Besides, Altintas et al., (2018) reported a microfluidic biosensor for real-time and automatic detection of *Escherichia coli* using electrochemical amperometry to achieve a lower detection limit of 50 CFU/mL. With more and more popularity of smartphones, they have been often applied to develop rapid and in-field detection assays of foodborne pathogens due to their portability, low cost and easy operation (Jing et al., 2014; Zangheri et al., 2015). Shrivastava et al., (2018) developed a smartphone-based fluorescent biosensor for detection of *Staphylococcus aureus* in a culture-free, rapid and quantitative manner from minimally processed liquid samples using aptamer functionalized fluorescent magnetic nanoparticles, and the lower detection limit of 10 CFU/mL could be obtained by counting the bacteria on the static fluorescence images. Cui et al., (2018) proposed a digital standard plate count method based on micro-droplet turbidity imaging and it could detect living bacteria in infant milk powder as low as 100 CFU/mL within 6 h. To the best of our knowledge, all the existing smartphone based biosensing methods are based on static image analysis, which are very difficult to realize on-line detection of foodborne bacteria (Wang et al., 2016; Zheng et al., 2019).

Zhu et al., (2011)reported a similar method using fluorescent labeling and smartphone video processing for cell counting. The authors used white blood cells in human whole blood as research model and integrated optofluidic fluorescent microscopy and flow cytometry on a smartphone. However, this proposed system could not be directly used for counting bacteria since the size of human white blood cell (10–12 μm) is much larger than foodborne bacteria (1–2 μm), which need higher resolution and larger magnification. More importantly, the video was post processed, indicating that this proposed system was not suitable for online identification. Therefore, further study based on this method might provide a promising way to detect single bacterium.

In this work, we developed a novel microfluidic biosensor for on-line, rapid and sensitive detection of *Salmonella* typhimurium combining immunomagnetic separation, fluorescence labeling and smartphone video processing. As shown in Scheme 1, the magnetic nanoparticles (MNPs) modified with the monoclonal antibodies (MAbs) against *Salmonella* typhimurium were first used to separate the target bacteria from the sample background to form the MNP-bacteria complexes (magnetic bacteria), which then reacted with the fluorescent microspheres (FMSs) modified with the polyclonal antibodies (PAbs) against *Salmonella* typhimurium to form the MNP-bacteria-FMS complexes (fluorescent bacteria). After the fluorescent bacteria were magnetically separated to remove the unbound FMSs, efficiently concentrated in small volume of PBS and continuously injected into the microfluidic chip, the smartphone based fluorescent microscopic system was used with the LED light source for fluorescent excitation to monitor the flowing fluorescent spots and the amount of the fluorescent bacteria was calculated through real-time video processing.

2. Materials and methods

2.1. Materials

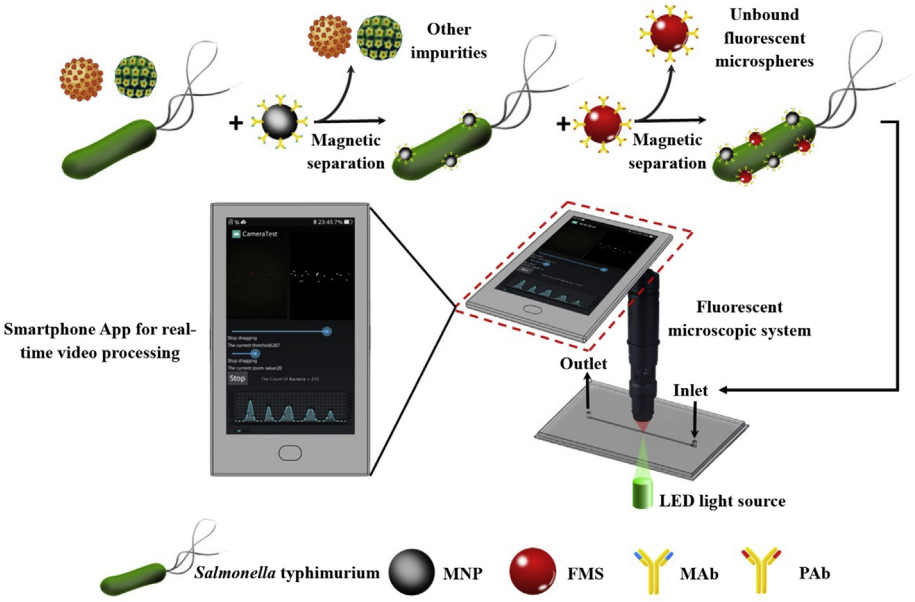
Salmonella typhimurium (ATCC 14028) was used as target bacteria, and *Listeria monocytogenes* (ATCC 13932), *E. coli* O157:H7 (ATCC 43888) and *Vibrio parahemolyticus* (ATCC 17802) were used as non-target bacteria. The MAbs (1 mg/mL) against *Salmonella* typhimurium from Meridian (Memphis, TN, US) and the PAbs (2.5 mg/mL) against *Salmonella* typhimurium from Fitzgerald (Acton, Massachusetts, US) were used for immunological reaction with *Salmonella* typhimurium. The biotin labeling kit from Elabscience (Wuhan, China) was used for the modification of biotin onto the MAbs. The streptavidin modified MNPs (1 mg/mL) with the diameter of 150 nm from Ocean Nano (MHS-150-10, Dunedin, FL, US) were used for immunomagnetic separation of *Salmonella* typhimurium. The carboxylate FMSs (10 mg/mL) with the diameter of 150 nm from VDO Biotech (FR150C, excitation wavelength: 535 nm, emission wavelength: 610 nm, Suzhou, China) were used for labeling *Salmonella* typhimurium as detection signal. N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl) was used for modifying the FMSs with the PAbs. Phosphate buffered saline (PBS, 10 times concentrated, pH 7.4) from Sigma-Aldrich (St. Louis, MO, US) was 10-fold diluted with deionized water to prepare the buffer solution. Tween-20 from Amresco (Solon, OH, US) was used for washing. Bovine serum albumin (BSA) from Sigma Aldrich was used for blocking. Brain heart infusion (BHI) medium from Remel (Lenexa, KS, US) and Luria-Bertani (Lee et al.) medium (Aoboxing Biotech, Beijing, China) fluorescen were used for bacterial culture. Deionized water produced by Advantage 10 (18.2 MΩ cm, Billerica, MA, US) was used for preparing all the solutions.

2.2. Fabrication of the microfluidic detecting chip

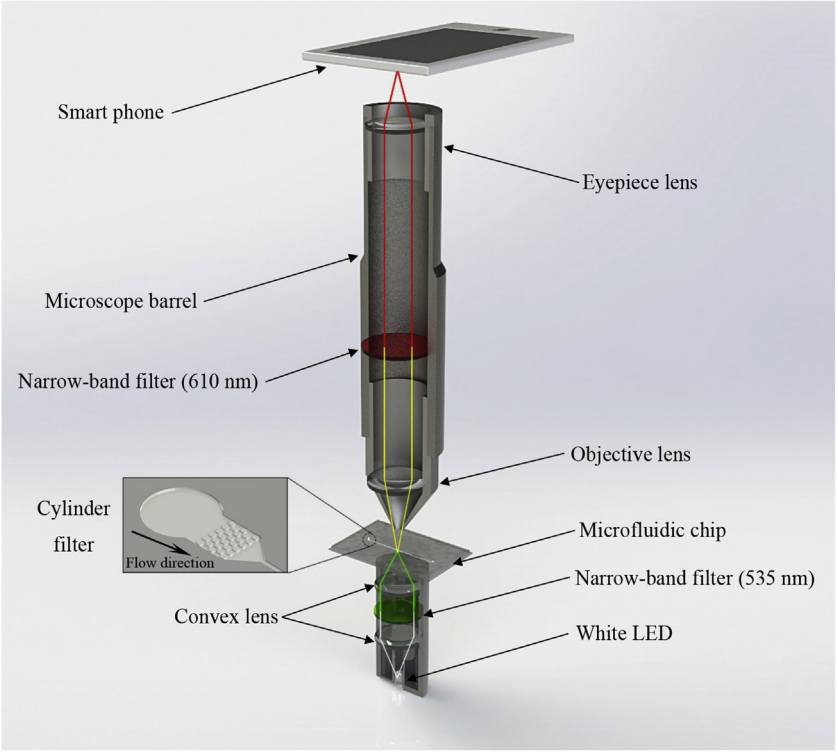
The microfluidic detecting chip is the key component of this proposed biosensor. It composed of two parts: (1) a fan-shaped cylinder filter for avoiding the blocking of the chip by the large-sized particles in the sample and enhancing the dispersion of the fluorescent bacteria, and (2) a line-shaped microchannel for observing and counting the fluorescent spots. As shown in Scheme 1(b), the cylinder filter had five rows of staggered micro-cylinders with a diameter of 50 μm, a height of 25 μm and an interval of 50 μm, and the straight microchannel had a width of 25 μm, a height of 25 μm and a length of 50 mm. The mold of the microfluidic channel was fabricated using SU8 photolithography. After the prepolymer and the curing agent from Dow Corning (Sylgard 184, Midland, US) were mixed at the ratio of 10:1 and used to make the poly (dimethoxy) silane (PDMS) microchannel, the microchannel and the glass plate from Sail Brand (7101, Jinan, China) were processed by surface oxygen plasma (Harrick Plasma, Ithaca, NY, US) and bonded to fabricate the microfluidic chip. The photo of the microfluidic chip is shown in Fig. S1.

2.3. Development of the smartphone based fluorescent microscopic system

The fluorescent microscopic system is another key component of this proposed biosensor. It composed of three parts: (1) the light source for fluorescent excitation, (2) the fluorescent microscope for optical amplifier, and (3) the smartphone App for real-time video processing. The structure of the fluorescent microscopic system is shown in Scheme 1(b). The light source with the wavelength of 535 nm was obtained using a 5 W white LED from Rongcheng Microelectronics (KB0603,



(a)



(b)

Scheme 1. (a) The principle of the proposed microfluidic biosensor for on-line, rapid and sensitive detection of *Salmonella* typhimurium; (b) The structure of the smartphone based fluorescent microscopic system.

Shenzhen, China) to provide the light beam, two convex lenses with the focus length of 50 mm from Yajiamei optics (YJM-50, Shenzhen, China) to focus the diverging beam, a narrow-band optical filter with the central wavelength of 535 nm from Hoda lens optics (D20-BPOD3-535, Shenzhen, China) to allow the penetration of the light with the wavelength of around 535 nm, and a 3D-printed holder to precisely fix these optical components.

The fluorescent microscope was constructed using flat-field achromatic objective lens with the magnification of 40 times, the numerical aperture of 0.65 and the working distance of 0.53 cm, a large flat-field coated eyepiece lens with the magnification of 25 times, the focus length of 12.5 mm, the view field of Φ6.5 mm, a narrow-band optical filter with the central wavelength of 610 nm and a 3D-printed holder to precisely fix these optical components. Both the objective lens and the eyepiece lens were purchased from Jiangnan Yongxin Optics (Nanjing, China) with the total magnification of 1000 times. The optical filter was purchased from Hoda Lens Optics (D20-BPOD3-610) and installed between the eyepiece lens and the objective lens to only allow the

penetration of the light with the wavelength of around 610 nm, which was the same with the emission wavelength of the FMSs. The fluorescent image or video were converged on the smartphone's CMOS sensor. The light source, the microfluidic chip, the fluorescent microscope and the smartphone were precisely aligned from bottom to top with appropriate spaces.

The smartphone App was developed under the Android operating system installed on a low-cost smartphone (Honor 9, Huawei, Shenzhen, China) with 4 GB RAM, 2.4 GHz CPU and 20 million pixels camera. The light source was first used to continuously excite the fluorescent bacteria to emit the fluorescent signals when they flowed through the microchannel. The video stream of the microchannel was then obtained from the camera and extracted into a sequence of grayscale frames. Finally, two adjacent frames were compared using the inter-frame difference algorithm to obtain the binarized difference images, and the flowing fluorescent spots could be accurately identified in the difference images by setting an appropriate threshold and counted to determine the total amount of the bacteria. The App is shown in Supplemental Video S1. The detailed dimension of the fluorescent microscopic system and the interface function description of the App are shown in Fig. S2 and Fig. S3, respectively.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.bios.2019.111333.

2.4. Modification of the FMSs with the PAbs

First, 20 μg of the FMSs and 68 μg of the PAbs against *Salmonella* typhimurium were added into 3 mL of PB buffer (pH 6.0, 10 mM) and stirred 30 min for better electrostatic adsorption. Then, 40 μg of EDC was added and stirred at room temperature for 30 min. This step was repeated 3 times to allow complete reaction between the PAbs and the carboxyl groups on the surface of the FMSs. Finally, 80 mg of BSA and 120 μg of EDC were added and stirred at room temperature for 60 min to minimize non-specific adsorption, followed by centrifuging at 10,000 rpm for 15 min to remove the surplus BSA and resuspending in 1 mL of PB buffer (pH 7.4, 10 mM) containing 5% sucrose and 0.02% sodium azide. The immune FMSs with the concentration of 0.2 mg/mL were stored in 4 °C for future use. To verify the successful synthesis of the immune FMSs, the dynamic light scattering analysis was conducted and the results are shown in Fig. S2.

2.5. Separation and detection of Salmonella typhimurium in pure culture

The target bacteria were first separated with the immune MNPs and then labelled with the fluorescent FMSs. Prior to test, the streptavidin modified MNPs and the biotinylated MAbs were mixed for 45 min to form the immune MNPs with the concentration of 1 mg/mL. Then, 20 μg of the immune MNPs were incubated with the bacterial samples with different concentrations ranging from 1.4×10^1 to 1.4×10^6 CFU/mL for 45 min to form the magnetic bacteria, respectively. After magnetic separation for 2 min to remove the background and washing with 500 μL of PBST (PBS with 0.05% Tween 20), 4 μg of the immune FMSs were incubated with the magnetic bacteria for 45 min to form the fluorescent bacteria. Finally, after magnetic separation for 2 min to remove surplus FMSs and washing with 500 μL of PBST, the fluorescent bacteria were concentrated in 50 μL of PBS.

The fluorescent bacteria were detected using the microfluidic chip and the fluorescent microscopic system. The fluorescent bacteria were continuously injected into the microfluidic chip using a precise syringe pump (Pump 11 elite, Harvard Apparatus, Holliston, MA, US). The light source with the wavelength of 535 nm was used to excite the FMSs on the fluorescent bacteria. The smartphone App was used with the developed fluorescent microscopic system to monitor and count the fluorescent spots when they were flowing through the microchannel, and the mathematical model of this biosensor was established by plotting the number of fluorescent spots with the concentration of the bacteria.

2.6. Detection of Salmonella typhimurium in apple juice

Apple juice was purchased from a local supermarket to simulate the real food sample. According to China's national standards on detection of Salmonella in foods (GB 4789), 25 mL of apple juice sample were first mixed with 225 mL of sterile PBS and homogenized for 2 min. Then, different concentrations of the target bacteria were added into 1 mL of the mixture, respectively, to obtain the spiked sample containing the target bacteria with the concentration from 1.4×10^1 to 1.4×10^6 CFU/mL. After that, each spiked sample was magnetically separated using the immune MNPs to form the magnetic bacteria, followed by being labeled with the immune FMSs to form the fluorescent bacteria. Finally, the fluorescent bacteria were detected using the microfluidic chip and the fluorescent microscopic system under the optimal conditions to obtain the concentration of the bacteria.

3. Results and discussions

3.1. Continuous counting of the fluorescent spots

Accurate counting of the fluorescent spots is crucial to the development of this proposed biosensor. The feasibility of this proposed fluorescent microscopic system for counting the fluorescent spots was first confirmed. The resolution ($\boldsymbol{d}$) of the microscopic system can be calculated using the following equation:

$$\boldsymbol{d} = 0.61 \times \boldsymbol{\lambda}/\mathrm{N_a} \quad (1)$$

where, $\boldsymbol{\lambda}$ is the wavelength of the emitting light, and $\mathrm{N_a}$ is the numerical aperture of the objective lens, i.e., the resolution of this microscopic system was calculated to be around 572 nm, which was much smaller than the size of the fluorescent bacteria (1–3 μm). Thus, this fluorescent microscopic system was able to identify a single fluorescent bacterium.

To further evaluate the performance of this proposed fluorescent microscopic system, its field depth was also confirmed. The field depth ($\boldsymbol{t}$) of this microscopic system can be calculated using the following equation:

$$\boldsymbol{t} = (\boldsymbol{n} \times \lambda)/(2 \times \mathrm{Na}^2) + (1000 \times \boldsymbol{n})/(\boldsymbol{M} \times \mathrm{Na} \times 7) \quad (2)$$

where, $\boldsymbol{n}$ is the refractive index between the objective lens and the sample, and $\boldsymbol{M}$ is the total magnification of the microscope, i.e., the field depth of this microscopic system was calculated to be around 1 μm, indicating that this microscopic system was able to observe and precise count 1/25 of the total fluorescent spots when they were flowing through the microchannel since the total depth of the channel was 25 μm.

For online identification and counting of the fluorescent spots, the inter-frame difference method was used to identify the flowing fluorescent spots in the difference images and the threshold for image binarization plays an important role. Thus, different grayscale thresholds ranging from 10 to 60 were applied to identify the fluorescent spots in a 2-min-long video of *Salmonella* typhimurium at the concentration of 1.4×10^5 CFU/mL in the microfluidic channel. The exact number of the fluorescent spots was counted by naked eyes to be 62. As shown in Fig. 1, when the thresholds were between 10 and 30, the results for counting the fluorescent spots were around 60 and very close to the actual number. However, when the thresholds were between 40 and 60, the results ranged from 17 to 1 and were obviously different from the actual number. Besides, when the thresholds were less than 10, the fluorescent spots were not identified probably due to the background noises and light field changes. Thus, the optimal threshold of 20 was used in this study.

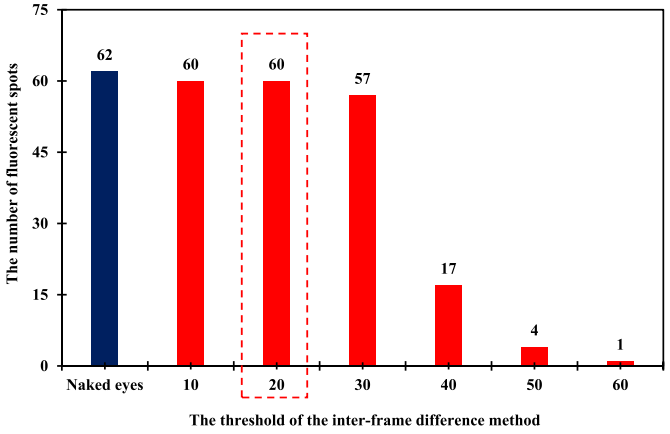


Fig. 1. Optimization of the threshold for the inter-frame difference method.

3.2. Optimization of the immune FMSs

In this study, the immune FMSs were used to label the bacteria for quantitative detection of the target bacteria. The amount of the FMSs on each bacteria has great impact on accurate identification of the bacteria. Therefore, different amounts of the immune FMSs were used to label *Salmonella* typhimurium at the concentration of 1.4×10^5 CFU/mL. After the fluorescent bacteria were injected into the microfluidic chip at the flow rate of 20 μL/h, they were continuously monitored and counted for 15 min using the smartphone App and the fluorescent microscopic system. As shown in Fig. 2, when the amount of the FMSs increased from 0.5 μg to 4.0 μg, the number of the fluorescent spots was counted from 170 to 450 and had only a little increase to 464 when the amount of FMSs was 8.0 μg. However, the theoretical number of the bacteria was ~560, which was around 100 more than the highest counted number. This probably was due to the following reasons: (1) the fluorescent microscopic system could only focus on one focal plane, (2) some target bacteria were lost during magnetic separation; (3) some target bacteria were not labeled with sufficient FMSs resulting in unsuccessful identification of the fluorescent bacteria; (4) some fluorescent bacteria were aggregated into larger ones; and (5) some fluorescent bacteria were not identified due to the relatively high flow rate. Thus, the optimal amount of 4.0 μg for the FMSs was used in this study.

3.3. Optimization of the flow rate and the detection time

In this study, the flow rate of the fluorescent bacteria and the detection time for counting the fluorescent bacteria have great impact on the accuracy and sensitivity of this proposed biosensor. Different flow rates ranging from 5 μL/h to 100 μL/h were applied to inject the fluorescent bacteria into the microfluidic chip for 15 min to count the amount of the target bacteria using the smartphone App. As shown in Fig. 3(a), when the flow rate changed from 5 μL/h to 20 μL/h, the number of the fluorescent spots was counted to increase from 272 to 870. However, when the flow rate kept increasing to 100 μL/h, the number of the fluorescent spots did not obviously increase, which might be mainly due to the processing limitation of the video stream using the smartphone. Thus, the optimal flow rate of 20 μL/h was used in this study.

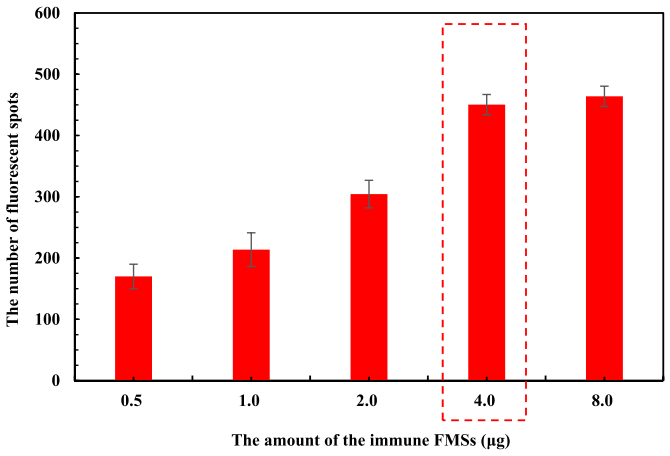
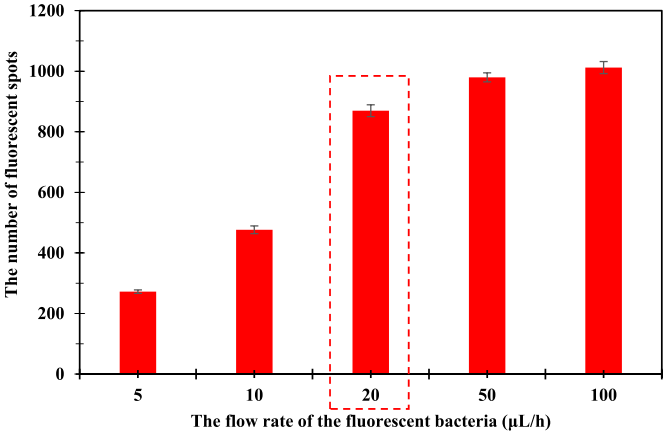
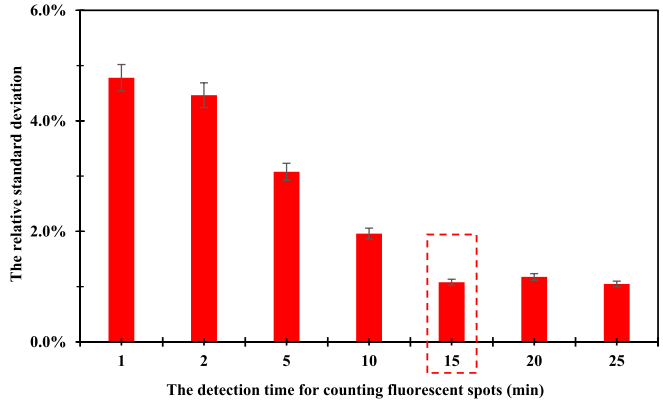


Fig. 2. Optimization of the amount of the immune FMSs.



(a)



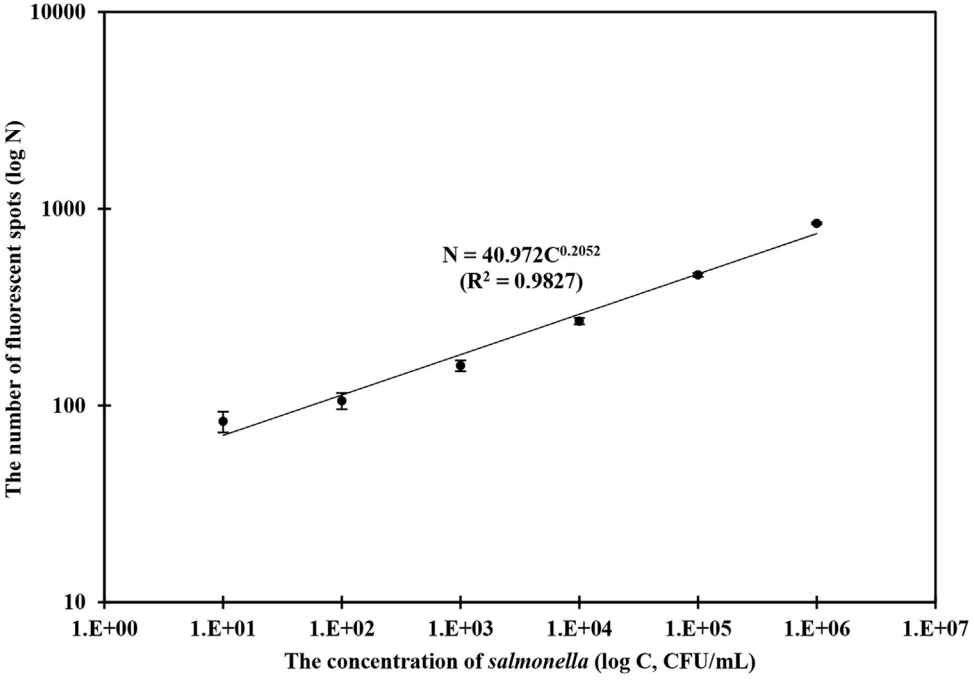
(b)

Fig. 3. (a) Optimization of the flow rate; (b) Optimization of the detection time.

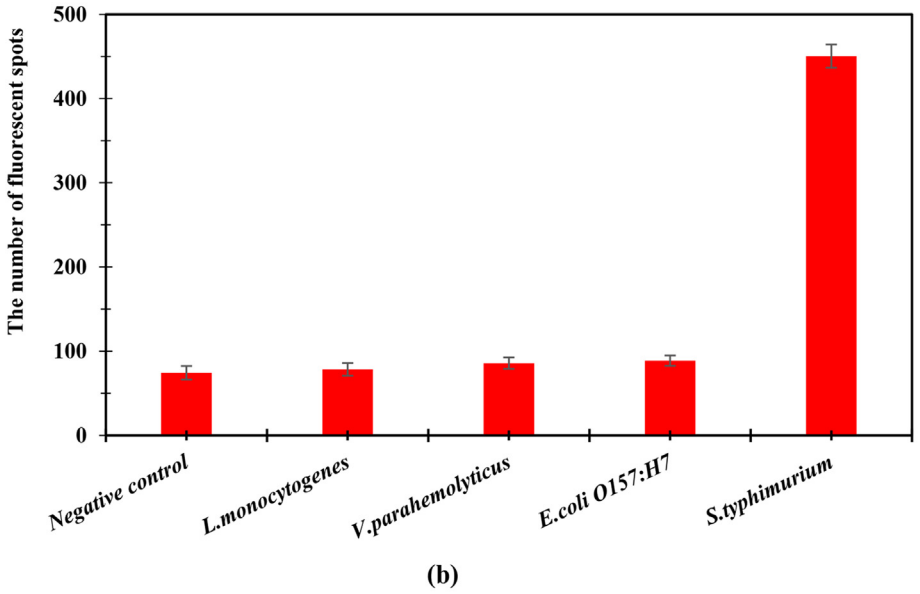
Besides, different detection times were applied to count the fluorescent bacteria using the smartphone App when they were flowing through the microfluidic chip at the flow rate of 20 μL/h for 15 min. The relative standard deviation of three parallel tests on the same concentration of the fluorescent bacteria was used to optimize the detection time. As shown in Fig. 3(b), when the detection time changed from 1 min to 15 min, the relative standard deviation decreased from 4.8% to 1.1%, and when the detection time was increased to 25 min, the relative standard deviation remained the same level. Thus, the optimal detection time of 15 min was used in this study.

3.4. Detection of Salmonella typhimurium using the proposed biosensor

To detect an unknown concentration of *Salmonella* typhimurium in a sample, the calibration curve of this biosensor was established. The target bacteria were first cultured in 5 mL of LB liquid medium at 37 °C for 12–16 h. Then, each culture was serially diluted using the sterile PBS to obtain the bacteria with the final concentrations from 1.4×10^1



(a)



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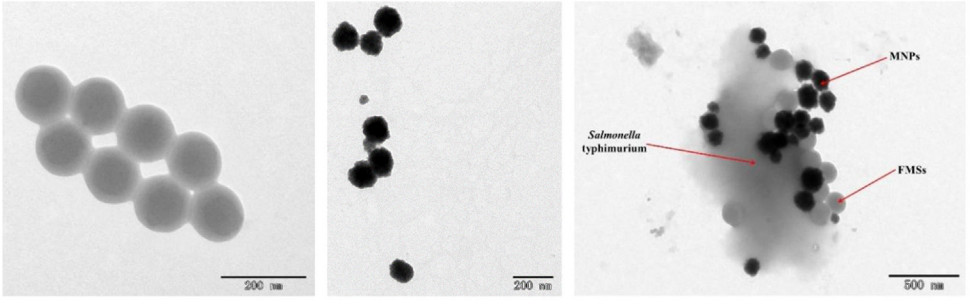

(c) (d) (e)

Fig. 4. (a) Calibration curve of this proposed biosensor; (b) The specificity of the proposed biosensor to the non-target bacteria; (c) The TEM image of the FMSs; (d) The TEM image of the MNPs; (e) The TEM image of the fluorescent bacteria.

Table 1
Detection of *Salmonella* typhimurium in apple juice using the proposed biosensor.

No.	The concentration of the spiked bacteria (CFU/mL)	The number of the fluorescent spots in spiked samples	The detected concentration of bacteria (CFU/mL)	Recovery (%)	CV (%)
1	1.4×10^2	98	126	90.00	6.38
2	1.4×10^3	167	1257	89.79	2.97
3	1.4×10^4	269	13546	96.76	2.93
4	1.4×10^5	477	137746	98.39	2.86
5	1.4×10^6	875	1187480	84.82	2.17

to 1.4×10^6 CFU/mL. Finally, different concentrations of *Salmonella* typhimurium ranging from 1.4×10^1 to 1.4×10^6 CFU/mL were detected at the optimal conditions using the proposed biosensor. As shown in Fig. 4(a), when the concentration (***C***) of *Salmonella* changed from 1.4×10^1 to 1.4×10^6 CFU/m, the number (***N***) of the fluorescent spots increased from 83 to 844. A good exponential relationship between the number and the concentration was found and could be expressed as:

$$\boldsymbol{N} = 40.97 \times \boldsymbol{C}^{0.21} \quad (3)$$

Based on three times of signal-to-noise ratio, the lower detection limit of this proposed biosensor was calculated to be 58 CFU/mL. The high sensitivity of this biosensor was attributed to the following aspect: (1) the smartphone App based on the inter-frame difference algorithm for accurate and online counting of the fluorescent spots; (2) the fluorescent microscopic system with optical filters for significant amplification of the fluorescent bacteria at a low background noise level; and (3) the cylinder filter at the entry of the microfluidic chip for better dispersion of the fluorescent bacteria and less interferences from the sample background.

To evaluate the specificity of this proposed biosensor, *Listeria monocytogenes*, *E. coli* O157:H7 and *Vibrio parahaemolyticus* were used as non-target bacteria. Both the target bacteria and the non-target bacteria at the same concentration of 10^5 CFU/mL and three negative controls were detected using the proposed biosensor. As shown in Fig. 4(b), the number of the target bacteria was 450, which was much larger than those of the non-target bacteria (70–90), indicating that the proposed biosensor has a good specificity. This is due to the good specificity of the MAbs for separation of *Salmonella* typhimurium and the PAbs for labeling of *Salmonella* typhimurium. Besides, as shown in Fig. 4(c)-(e), transmission electron microscopy (TEM) was used to characterize the MNPs and the FMSs and verify the successful forming of the fluorescent bacteria.

Finally, the proposed biosensor was compared with some previously reported smartphone-based biosensors, and the result was shown in Table S1.

3.5. Detection of Salmonella typhimurium in the spiked apple juice

To further evaluate the applicability of this proposed biosensor for Salmonella in real samples, apple juice purchased from local supermarket was used and pretreated based on China's food safety national standards. The spiked samples were prepared using the method described in section 2.6 and finally detected using this proposed biosensor under the optimal conditions. All the samples were parallel tested three times, and all the data were the average of the three experiments. The recovery of the spiked bacteria was calculated as the ratio of the detected concentration of bacteria to the concentration of the spiked bacteria. As shown in Table 1, the recoveries for different concentrations of Salmonella in apple juice ranged from 84.82% to 98.39% with an average recovery of 92.64%, indicating that the proposed biosensor was applicable for detection of *Salmonella* in real samples.

4. Conclusions

In this study, we successfully developed a microfluidic biosensor for online, rapid and sensitive detection of *Salmonella typhimurium* using immunomagnetic separation, fluorescent labeling, microfluidic chip and smartphone video processing. Under the optimal conditions, the proposed biosensor was able to detect *Salmonella typhimurium* as low as 58 CFU/mL within 2 h, and has shown great potential for on-line monitoring of foodborne pathogens. At present, this biosensor is mainly limited by the video processing speed and image capturing quality. The sensitivity of this biosensor can be further improved using brighter fluorescent materials to label the bacteria for amplification of the fluorescent signals and higher-performance smartphones or devices with faster CPU speed and higher camera resolution to process the video for more accurate and faster counting of the fluorescent bacteria. Besides, online and accurate detection of single bacterium can be expected to realize by combining this biosensing method with microfluidics.

CRediT authorship contribution statement

Siyuan Wang: Software, Data curation, Writing - original draft. **Lingyan Zheng:** Methodology, Formal analysis. **Gaozhe Cai:** Validation, Visualization. **Ning Liu:** Project administration, Funding acquisition. **Ming Liao:** Project administration, Funding acquisition. **Yanbin Li:** Project administration, Funding acquisition. **Xibin Zhang:** Project administration, Funding acquisition. **Jianhan Lin:** Conceptualization, Supervision, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.111333.

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