Immunomagnetic separation and rapid detection of bacteria using bioluminescence and microfluidics

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ABSTRACT

This paper describes an immunomagnetic separation of target bacterial cells from others by using magnetic bead. The surface of bead was coated with antibodies which can capture specific organism. The binding efficiency of immunomagnetic bead (IMB) capturing target bacterial cells was higher than 98% when the concentrations of target and interferent bacterial cells were at the same level. The concentration of bacteria was determined indirectly by detecting adenosine 5'-triphosphate (ATP) employing bioluminescence (BL) reaction of firefly luciferin-ATP. Benzalkonium chloride (BAC) was used as an ATP extractant from living bacterial cells. We found that BAC could enhance the light emission when the concentration of BAC was less than $5.3 \times 10^{-2}\%$ (w/v) and the BL intensity reached its maximum at the concentration of BAC was $2.7 \times 10^{-2}\%$, which was 10-fold stronger than that without BAC. Based on the principle of the IMB, a microfluidic chip combined with immunofluorescence assay for separating and detecting bacteria simultaneously was also developed. The IMBs were magnetically fixed in the bead-beds of chip channels with a 3-mm diameter of NdFeB permanent magnet. The target bacterial cells can be captured magnetically and observed by a fluorescent microscope.

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1. Introduction

Rapid detection of bacteria is necessary for the safety of food products, the veracity of clinical diagnostics, the future progress of gene engineering, the prohibition of threat of possible biological warfare, etc. [1,2]. Previously, the microbiological methods, containing replication bacterial cells to 10^9 cell/mL, specific identification on selective media and biochemical tests, provided us a traditional way to detect bacteria, which usually cost several days [3]. So a truly rapid and sensitive monitoring system for detecting and identifying bacteria is in great demand. Adenosine 5'-triphosphate (ATP) is present in all living cells and the amount of it per cell remains fairly constant ($\sim 10^{-18}$ mol/cell) [4]. Therefore, an assay of ATP is responsible for the concentration of bacterial cells. Firefly bioluminescence (BL) is routinely used for the detection of ATP due to the number of photons (BL intensity) is directly proportional to the concentration of ATP [5–7], which can be measured at femtomole levels using the luciferin-ATP reaction [8].

In the assay of intracellular ATP from bacterial cells, ATP extractants are used for releasing ATP from living bacterial cells. Plenty of choices in ATP extractant are available, such as trichloroacetic acid (TCA), Triton X-100, ethanol, and benzalkonium chloride (BAC). Among ATP extractant, BAC is widely used because it can extract ATP from more living cells especially bacteria [9]. Previously, it is reported that BAC inhibits the activity of luciferase besides releasing ATP in the BL reaction; the way to eliminate the inhibitory effect is to dilute the extracts containing intracellular ATP and BAC, thus resulting in lowering of sensitivity for determining ATP. Another method to eliminate the inhibitory effect, which does not induce a decrease in determining ATP, is to use liposome, composed of phosphatidylcholine and cholesterol, the function of which in BL reaction is achieved by associating with BAC before BAC inhibiting luciferase [10–12]. However, there are some disadvantages by introducing liposome: the procedure is too complex; it costs too much time in synthesizing satisfied liposome for the BL reaction; the improved degree in sensitivity by the liposome is limited. So it is necessary to establish a more convenient method to improve the sensitivity in the BL reaction. According to experiments, we found that when the concentration of BAC was less than some value, BAC itself could enhance the BL intensity, which means that it does not need other reagents to improve the sensitivity in the BL reaction.

The immunomagnetic bead composed of magnetic particle core inside and polymer group conjugated by antibodies on the sur-

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Fig. 1. The illustration of IMB used in experiments. (A) The schematic mechanism of synthesizing IMB anti-*S.aureus*. (B) The structure of IMB. (C) Schematic illustration of the immunomagnetic separation process for separating *Salmonella* in a tube of the bioluminescence method. Scale bar = 10 μm .

face, are designed for immunomagnetic separation (IMS) [13]. Typically, the beads are 1–5 μm spheres containing $\gamma\text{-Fe}_2\text{O}_4$, MeFe_2O_4 (Me=Co, Mn, Ni), and Fe_3O_4 to make them superparamagnetic in the presence of magnetic field. The antibody coated on the surface of the IMB could capture the target organism when they are close to IMB. So IMS is a rapid, specific, efficient and technically simple method that can be used for separation of target organism directly from non-target organism and other particles without any need for centrifugation or filtration [14–16].

Microfluidic systems, an attractive platform for rapid few cells functional analysis, appear to offer a powerful means to automate and miniaturize sample preparation for bioassays [17–19]. On-chip assays using micron-sized fluidic channels have demonstrated the ability to perform rapid and sensitive biological assays. In addition, the small dimensions of microdevices allow for the creation of portable analytic devices. Magnetic bead-beds within a microfluidic device was proposed for capturing cells in order to obtain a more controllable and compact magnetic bed, which can improve the capture efficiency. Furdulj [20] reported a similar approach, in which the detection results were obtained by the PCR step. However, the PCR step is time consuming and off-line. So we developed an on-chip immunofluorescence assay for bacteria analyzing using microfluidic chip combined with the bacterial cells capture of IMB. Before introducing into microchip, bacterial cells were firstly mixed with the FITC-labeled antibodies, which supported an easily way to detect by charge coupled devices (CCD) in the microscope. Separation is achieved by bacteria–FITC-labeled antibodies complex captured by bead-beds in the chamber of microchannel [21], and

detection is obtained by observing the fluorescent phenomena from the bead-beds.

In this paper, the optimum conditions on separating and detecting bacterial cells based on bioluminescence and microfluidics were studied. *Salmonella* (CGMCC 1.1552) and *Staphylococcus aureus* (*S. aureus*, CGMCC 1.363) were used as the test target bacteria, which were chosen to form a model system due to the ready availability of immunoassay reagents and they are the typical ones of gram positive and negative bacteria. Results from this analyte can be generalized and extended to other bacterial species by changing IMB and antibodies.

2. Experimental

2.1. Chemical and reagents

Salmonella (CGMCC 1.1552) and *Staphylococcus aureus* (*S. aureus*, CGMCC 1.363) are from China General Microbiological Culture Collection Center (Beijing, China). The magnetic beads were purchased from Invitrogen Dyanal (Oslo, Norway) and its diameter is 2.8 μm in average. Luciferase from firefly (*Photinuspyralis*) and D-luciferin were obtained from Promega (Madison, USA). Benzalkonium chloride (BAC, Predominantly $\text{C}_{12}\text{H}_{25}\text{N}(\text{CH}_3)_2\text{C}_7\text{H}_7\text{Cl}$, also contains C_{12} and C_{16} homologues) was purchased from Sigma–Aldrich Co. (USA). Adenosine 5'-triphosphate disodium salt (ATP) and magnesium chloride were obtained from Amresco (Ohio, USA). EDTA, dithiothreitol, bovine serum albumin (BSA), D-glucose, and 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (Hepes) were obtained from Genview (Texas, USA). Yeast extract and tryp-

Fig. 2. The schematic of the microfluidic device. (A) The depth and width of the microchip. (B) Layout of the U-type microchip used for separation and detection of bacteria. Dimensions (in mm) correspond to the photomask features. Reservoirs A–B, C–D and E represent the inlets of IMB, buffer solutions and bacterial sample, respectively, and F is the outlet. (C) The appearance of the PDMS/glass microfluidic chip. (D) IMB is pumped to the channel for capture with a magnet. (E) The sample (bacteria and FITC-labeled antibodies) is introduced from reservoir E. Beads show as blue and green streaks, sample as dark staff.

tone were purchased from Oxoid Ltd. (Basingstoke, Hampshire, England). Sodium chloride was obtained from Beihua Fine Chemicals Co., Ltd. (Beijing, China). Beef extract was purchased from Shuangxuan Microorganism Culture Factory (Beijing, China). IMB anti-*Salmonella* was obtained from Invitrogen Dynal (Oslo, Norway). FITC-labeled anti-*Salmonella* antibody, FITC-labeled anti-*S. aureus* antibody and anti-*S. aureus* antibody were purchased from Abcam (Cambridge, UK).

A 10.0 mM solution of ATP was daily prepared by dissolving the compound with the “buffer A” (25 mM Hepes and 2.0 mM EDTA, pH 7.6). Working solution was prepared by serial dilution with the “buffer A”. A 1.0% (w/v) solution of BAC was prepared by dissolving the compound with the “buffer A”, and stored at 4 °C. A luciferase solution was prepared by dissolving 50 mg of luciferase in 10 mL of “buffer L” (25 mM Hepes, 2.0 mM EDTA, 20 mM magnesium chloride, 1.2 mg/mL BSA, and 2.0 mM dithiothreitol, pH 7.6); a luciferin solution was prepared by dissolving 5 mg luciferin in 5 mL with the “buffer L”. The luciferase and luciferin solution were stored in portions of 200 μL and 100 μL at –20 °C, respectively. When used, the two portions were mixed and 4.8 mL “buffer L” was added into the mixture. Other reagents were commercially available as analytical reagent or laboratory grade materials. All solutions were prepared with ultrapure deionized water from a UV ultrapure water system (EASYpure® II, Barnstead International, USA).

The multilite luminometer containing the equipment of chemiluminescence and UV detection was obtained from Biotech

(Synergy 4, Vorment, USA). The microscope (DMI 4000) and the fluorescent source (CTR 4000) were purchased from Leica (Wetzlar, Germany).

2.2. Preparation of bacteria samples

The target organism was *Salmonella*; the interferent bacterial cell was *S. aureus*. They were grown 3–5 h at 37 °C in BPY culture (10 g/L tryptone, 5 g/L beef extract, 5 g/L yeast extract, 5 g/L D-glucose, and 5 g/L sodium chloride). The culture was grown to midlog phase ($OD_{600} \approx 0.6$). Optical density at 600 nm (OD_{600}) was measured with the multilite luminometer in a 96-well plate. The bacterial cells were collected by centrifugation at 10,000 rpm for 5 min at 4 °C by a centrifuge (centrifuge 5804 R, Eppendorf, USA). The pellet was suspended in 1.0 mL of the “buffer A” solution. Working cell suspensions were prepared by serially diluting with the “buffer A” solution. All bacteria suspensions were stored on a crushed ice bath until the extraction of ATP from bacterial cells.

For microfluidics, FITC-labeled anti-*Salmonella* antibody and FITC-labeled anti-*S. aureus* antibody were diluted with “buffer A” solution to reach the final dilutions of 1:40 and 1:10, respectively. The number of working bacterial cells in the process of serially diluting was 3, which meant the bacterial cells in the experiment had been diluted by 1000-fold. Then, the antibody and cells were mixed and shaken up for 5 min on a crash ice bath according to the relationship of antibody–antigen.

2.3. Bioluminescence detection

The BL experimental procedure consisted in pipetting a 100- μ L portion of the ATP (standard samples or intracellular extracts) solution into a well of 96-well plate in the multilite luminometer. Then a 50- μ L of portion of luciferase–luciferin mixture solution was injected into the well. Light output was integrated for 10 s, following a 2 s' delay (shaking), with readings presented as relative light units (RLU). Bacterial numbers in sample dilutions were determined as colony forming units (CFU) from triplicate plate counts.

For intracellular ATP assays, a 100- μ L portion of bacteria suspension was mixed with some portion of 1.0% BAC solution in a 2-mL plastic cuvette to extract ATP from bacterial cells. After incubating on a crashed ice bath for approximately 5 min, the extract was diluted with some portion of “buffer A” solution to make the final volume of mixture become 1.0 mL. The 100- μ L aliquot of the diluted extract was added into a well of 96-well plate, and then 50- μ L portion of luciferase–luciferin mixture solution was injected into the well, light output was measured after 2 s' shake in the multilite luminometer.

2.4. Immunomagnetic separation

IMB anti-*S. aureus* (Fig. 1A) was synthesized as the instructions of “Dynabeads® M-280 Tosylactivated”; the process was shown in Fig. 1A. Assays employing IMB anti-*Salmonella* (Fig. 1C) for separating *Salmonella* as showed in Fig. 1C were performed by taking 100- μ L portion of bacterial dilutions in a 2-mL tube and adding 50- μ L washed IMB anti-*Salmonella* dilution solution. These were mixed at room temperature using a Dynal MPC-1 sample mixer (Invitrogen Dynal AS, Oslo, Norway) for 10 min before being immobilized using a Dynal MPC magnet (Invitrogen Dynal AS, Oslo, Norway) for 2 min.

The immobilized beads were then washed with a 150- μ L portion of “buffer A” and re-suspended in 1 mL portion of “buffer A” after adding a 40- μ L portion of 1.0% BAC solution for bioluminescence assay.

2.5. Chip fabrication

Polydimethylsiloxane (PDMS)/glass microfluidic chip (Fig. 2C) was fabricated using the standard soft lithography method [22–25]. The microscale channels were designed using the Adobe Illustrator software and then printed out on a transparency by a high resolution puncher as shown in Fig. 2. The transparency was then used as the photomask in photolithography for the fabrication of a master on a silicon wafer. We used negative photoresist SU-8 2050 (MicroChem, Newton, MA, USA). PDMS (Silicone elastomer, Dow Corning, Midland Michigan, USA) prepolymer mixture consisting of monomer and curing agent with the mass ratio of 10:1 was poured on the SU-8/silicon wafer master and then cured at the temperature of 60 °C for 0.5 h. The peeled PDMS stamp (about 5 mm thick) with structures of the microchannels at the surface was then drilled six inlet and outlet holes. A glass slide (7105, Sail Brand, Jiangsu, China) was pre-cleaned in ($\text{H}_2\text{O}:\text{NH}_4\text{OH}$ (27%): H_2O_2 (30%) = 5:1:1, volumetric ratio) solution with stirring at around 70 °C for 1 h and then rinsed with pure water and dried. The PDMS chip and the glass slide were oxidized using a Plasma Cleaner (PDC-32G, Harrick, USA) in atmosphere for 1.5 min and then immediately put into contact to form the PDMS/glass microchip. The depth and width (Fig. 2A) of microchip were 40 μm and 60 μm , respectively.

For magnetic bead-beds, IMB for *Salmonella* and for *S. aureus* was introduced into microchannels from A and B inlet using a 250- μ L syringe (gastight #1725, Hamilton, Switzerland) by a pump (PHD 2000, Harvard, USA) for 10 min at 0.10 $\mu\text{L}/\text{min}$, respectively, and

Fig. 3. The optimized conditions on separating bacteria using bioluminescence. Effect of BAC concentrations on BL response curves. (A) The standard ATP, [ATP] = $\sim 10^{-10}$ M. (B) *Salmonella*, the cell number is $\sim 10^5$ CFU/mL. (C) *S. aureus*, the cell number is $\sim 10^5$ CFU/mL. (D) Effect of dilution fold of IMB on BL intensity. Cell numbers: 10^5 CFU/mL, [BAC] = $2.7 \times 10^{-2}\%$.

captured magnetically with a 3 mm diameter NdFeB permanent magnet (Yingke Hongye Technical Corp., Beijing, China), as shown in Fig. 2D.

2.6. Chip operating procedure

Bead-beds were prepared as described, as shown in Fig. 2E, then the sample 1 (*Salmonella*, FITC-labeled anti-*Salmonella* antibodies) was firstly pumped through the chip from E inlet using a 100- μ L syringe (gastight #1710, Hamilton, Switzerland) for 20 min at 0.10 μ L/min, simultaneously other inlets except F were pumped “buffer A” at 0.05 μ L/min in order to keep the sample flowing circularly in the U-type microchannel. Next, sample 2 (*S. aureus*, FITC-labeled anti-*S. aureus* antibodies) was introduced. The results were obtained by using the fluorescent microscope equipped with a digital camera under I3 filter set (470–490 nm).

3. Results and discussion

3.1. Effect of BAC on the BL reaction

To investigate the effect of BAC on the BL reaction, light emission was observed in standard ATP and intracellular ATP from *Salmonella* and *S. aureus* cells, respectively. The concentration of BAC was in the range of 6.7×10^{-3} to $6.7 \times 10^{-2}\%$ in both mixtures. The results, as shown in Fig. 3, indicated the BL intensity and the amount of light emission were remarkably enhanced with the concentration of BAC increasing until $2.7 \times 10^{-2}\%$, after that the BL intensity decreased rapidly. Besides that, the BL intensity was stronger than that without BAC until the concentration of BAC was up to $5.3 \times 10^{-2}\%$. At the maximum point, the BL intensity was improved by a factor of 10 times compared to that without BAC. The mechanism of firefly bioluminescence decomposition can be deduced as follows [26].

Firefly luciferin reacts with ATP under the catalysis of luciferase and Mg^{2+} , yielding adenyl luciferin, a mid-product (reaction (1)), which will be oxidation by O_2 according to nucleophilic acyl substitution, making CO_2 is left and yielding oxyluciferin (reaction (2)), which is in the excited state (marked as *) and unstable. Next oxyluciferin* decays to the ground state, accompanied with light as the energy of emission from the reaction as the form of photons (reaction (3)):

The reaction (1) is the rate-determining step (RDS) of the three reactions. So the above observations can be interpreted as follows. BAC, one of the cationic surfactants, the polar head of which can attract the anionic groups of luciferin and ATP, leads to accelerate the reaction (1) due to improve the combination of ATP and luciferin. Besides, BAC can inhibit the activity of luciferase. Therefore, before the concentration of BAC was up to $5.3 \times 10^{-2}\%$, the improvement from BAC could counteract its inhibitory effect, thus resulting the enhancement of BL intensity with increasing concentration of BAC. On the contrary, after the concentration of BAC was more than $5.3 \times 10^{-2}\%$, the inhibitory effect was dominant in the BL reaction, inducing the decrease of BL intensity.

The investigation in intracellular ATP from bacterial cells was shown in Fig. 3B (*Salmonella*) and Fig. 3C (*S. aureus*), which were examined by adding different volume of 1.0% BAC solution in a 100-

Table 1

The BAC concentrations in releasing ATP and BL reaction.

Portion of adding BAC (μ L)	Concentration of BAC in releasing ATP (w/v)	Concentration of BAC in BL reaction (w/v)
100 (1.0%)	0.50%	0.07%
90 (1.0%)	0.47%	0.06%
80 (1.0%)	0.44%	0.05%
70 (1.0%)	0.41%	0.05%
60 (1.0%)	0.38%	0.04%
50 (1.0%)	0.33%	0.03%
40 (1.0%)	0.29%	0.03%
30 (1.0%)	0.23%	0.02%
20 (1.0%)	0.17%	0.01%
100 (0.1%)	0.05%	0.01%
50 (0.1%)	0.03%	0.00%
20 (0.1%)	0.02%	0.00%

μ L of bacteria dilution. The volume of 1.0% BAC was in the range of 10–100 μ L, and the concentration of BAC in releasing ATP and BL reaction was shown in Table 1. Bacterial cell number of both is $\sim 10^5$ CFU/mL. The trend of both curves in Fig. 3B and C was as similar to Fig. 3A, which suggests: firstly, the reason for the enhancement and decrease of the BL intensity is from BAC instead of ATP; secondly, the activity of intracellular ATP and standard ATP was same in the BL reaction; finally, the efficiency of BAC releasing ATP is high. The BL intensity reached maximum when the concentration of BAC was $2.7 \times 10^{-2}\%$ in both Fig. 3A and B, which means the optimum concentration of BAC is $2.7 \times 10^{-2}\%$ in this method of detecting bacteria.

3.2. Measurement of ATP in bacterial extracts

The calibration curve for standard ATP and intracellular ATP shown in Fig. 4 was prepared under the optimum concentration of BAC. The detection limit for ATP and bacteria was defined as the concentration of ATP and cell numbers that produced the BL intensity equal to 3-fold of the blank intensity counted in the mixture containing no ATP or bacterial extracts. The detection limit of standard ATP in this method is 10^{-17} M and the linear range is 10^{-17} – 10^{-14} M. Correlation coefficient for linear regression analysis is indicative of small errors and excellent linear reliability ($r^2 > 0.996$). In applications, as shown in Fig. 4B and C, the detection limit of both bacteria is 10 CFU/mL and the linear range is 10^1 – 10^4 CFU/mL, which is better than previous reports. So using this method we can detect bacterial cells even when they are in very low concentration.

3.3. Immunomagnetic separation

The efficiency of IMB capturing target bacterial cells is examined by condition of dilution fold and selectivity, and the results were shown in Fig. 3D and Table 2, respectively. The concentration of bacterial cells (*Salmonella*) is $\sim 10^5$ CFU/mL, and incubation time is approximately 10 min. Then the IMB capturing bacteria was removed by employing magnetic field. Next BAC was added in the washed bacteria-IMB complex suspension solution on a crashed ice bath to release ATP. Finally, luciferin-luciferase solution was injected in the extracts and detection began after shaking. The results, in Fig. 3D, show the BL intensity increased and reached maximum when dilution fold was 6, after which BL intensity decreased with the dilution fold increasing. The reason for the increase in BL intensity is maybe due to the beads density decreases gradually with the dilution fold increasing, which results in the capacity of beads blocking releasing ATP is weaker than before. Later, that there are relatively fewer beads to capture bacteria with the dilution fold continuous increasing may well explain the decrease of BL inten-

Fig. 4. The results of detecting bacteria using bioluminescence. Linear range of standard and intracellular ATP concentrations. [BAC] = $2.7 \times 10^{-2}\%$. (A) Standard ATP's, $r^2 = 0.996$. (B) *Salmonella*'s, $r^2 = 0.999$. (C) *S. aureus*'s, $r^2 = 0.999$.

sity. So the optimum dilution fold of IMB is 6 in immunomagnetic separation.

The selectivity of the IMB was examined by using the *S. aureus* as an interferent for the *Salmonella* target cells. The colonies produced by the two species onto BPY agar could be easily identified and separately counted (*S. aureus*, yellow; *Salmonella*, white). The

results in Table 2 indicate no significant non-specific binding of *S. aureus*. However, the binding efficiency of the *Salmonella* was reduced in the presence of higher concentration of *S. aureus*. The experiments showed that, although not so much *S. aureus* cells were captured by the beads, there could have been non-specific binding of cell-free material, which appeared to interact with the antibodies on the beads with the effect of reducing the number of binding sites available to capture the *Salmonella* cells. Therefore, the selectivity of IMB in immunomagnetic separation is acceptable.

3.4. Microfluidic chip method

In order to make good use of IMB, magnetically captured bead-bed was designed in a microfluidic chip (see Fig. 2). Initial formation of the bead-bed is an important step in preparing the device for sample capture. Bead capture was maximized through the use of an asymmetric magnetic field, achieved by placing a magnet on only one side of the chip. In the U-type microchip, the chamber (the part of the channel expanding outwards) provided a place to form bead-beds, shown in Fig. 5A and B, illustrating the magnetic beads were almost totally captured by the outside magnet and there was no bead in the microchannel.

Fig. 5C–H showed that there was no affinity between IMB and FITC-labeled antibodies and IMB alone could not be observed by the fluorescent microscope.

Fig. 6C and D demonstrated that when sample 1 (*Salmonella* and FITC-labeled anti-*Salmonella* antibodies) was introduced through the microchannel, only the bead-beds composed of the IMB anti-*Salmonella* were observed, so did the bead-beds composed of IMB anti-*S. aureus* when sample 2 was introduced, shown in Fig. 6A and B. So it is suggested that the two kinds of bacterial cells were separated and detected simultaneously by using the U-type microchip.

3.5. Primary application for detecting bacterial cells

The optimized conditions for the separation and detection of target bacteria from interferent and others are described in Sections 2.2–2.4. To demonstrate the utility of this method, target bacterial cells (*Salmonella*) were measured with and without interferent bacterial cells (*S. aureus*). One sample only contained target bacterial cells and the other also contained interferent bacterial cells. Initially, the number of bacterial cells in the two samples was very low, then the bacterial cells in the two samples replicated at the same condition until the culture was grown to midlog phase ($OD_{600} \approx 0.6$) in 3–5 h. The two samples were separated by using IMB and extracted ATP by BAC. Finally, whether or not the original sample contained the target bacterial cells could be judged by comparing the BL signals in the reaction of the extracts and luciferase–luciferin solution, because the signal from the sample in the presence of target bacterial cells must be stronger than that without target bacterial cells. The results showed that the BL intensity with target bacterial cells was at least 100 times higher than that in the presence of interferent bacterial cells alone.

Table 2

Specific capture of *Salmonella* onto immunomagnetic bead from a mixed culture containing an excess of *S. aureus* cells.

<i>Salmonella</i> (CFU/mL) in suspension	<i>S. aureus</i> (CFU/mL) in suspension	<i>S. aureus</i> : <i>Salmonella</i> ratio	<i>Salmonella</i> (CFU/mL) on beads	<i>S. aureus</i> (CFU/mL) on beads
2.0×10^3	2.1×10^3	1.1:1	2.0×10^3 (98.5%)	1.3×10^0 (0.06%)
1.7×10^3	1.9×10^4	10.7:1	1.4×10^3 (76.4%)	2.1×10^1 (0.11%)

Fig. 5. The images of bead-beds and fluorescent microphotograph of IMB and FITC-labeled antibodies. (A) The left of the chamber placing bead-beds, showed there was no bead in the microchannel. (B) The right of the chamber, just close to the shadow cast by the magnet above. (C) The normal FITC-labeled antibodies. (D) The normal magnetic beads. (E) The normal magnetic beads after mixed with FITC-labeled antibodies. (F) The FITC-labeled antibodies exposed in the fluorescence. (G) The magnetic beads exposed in the fluorescence. (H) The magnetic beads after mixed with FITC-labeled antibodies exposed in the fluorescence. Scale bar = 10 μm .

Fig. 6. The results of separation and detection of bacteria using the microfluidic chip. (A) Normal bead-beds composed of IMB anti-*S. aureus*, in the right of the shadow cast by the magnet above. (B) Bead-beds composed of IMB anti-*Salmonella* was exposed in the fluorescence, showing the bead-beds captured *S. aureus*. (C) Normal bead-beds composed of IMB anti-*Salmonella*, under the shadow cast by the magnet above. (D) Bead-beds composed of IMB anti-*Salmonella* was exposed in the fluorescence, showing the bead-beds captured *Salmonella*. Scale bar = 10 μm .

4. Conclusions

We found that except for the inhibitory effect on luciferase, BAC can not only release ATP from bacterial cells, but also enhance the BL intensity at some range of concentration. In addition, high efficiency in separating bacterial cells can be achieved by employing immunomagnetic bead. As a result, a highly sensitive and rapid immune bioluminescence method was established for separating and detecting bacteria, which was applied to *Salmonella* in the presence of *S. aureus* as interferent. Furthermore, a U-type PDMS/glass microchip was developed to separate and detect bacteria simultaneously.

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