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Identification of pathogenic bacteria in human blood using IgG-modified Fe₃O₄ magnetic beads as a sorbent and MALDI-TOF MS for profiling

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Abstract

A method is described for fast identification of bacteria by combining (a) the enrichment of bacterial cells by using magnetite (Fe₃O₄) magnetic beads modified with human IgG (IgG@Fe₃O₄) and (b) MALDI-TOF MS analysis. IgG has affinity to protein A, protein G, protein L and glycans on the surface of bacterial cells, and IgG@Fe₃O₄. It therefore is applicable to the preconcentration of a range of bacterial species. The feasibility of the method has been demonstrated by collecting six species of pathogenic bacteria (Gram-positives: *Staphylococcus aureus* and *Kocuria rosea*; Gram-negatives: *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*). Bacteria with concentrations as low as 10 CFU·mL^{-1} in spiked water samples were extracted by this sorbent with recovery rates of >50%. After enrichment, bacteria on the IgG@Fe₃O₄ sorbent were further identified by MALDI-TOF MS. Bacteria in concentrations as low as 10^5 CFU in 100 μL of human whole blood can be identified by the method. Compared to other blood culture based tests, the culture time is shortened by 40% (from ~10 h to ~6 h), and the plate culture procedure (overnight) is avoided. After short blood culture, the enrichment and identification can be finished in one hour. The IgG@Fe₃O₄ is of practical value in clinical diagnosis and may be combined with other identification methods, e.g. PCR, Raman spectroscopy, infrared spectroscopy, etc.

Keywords Affinity enrichment · Mass spectrometry · Human blood · Bacterial infection · Clinical diagnosis

Introduction

Bacterial infection is a major cause for morbidity and mortality all over the world [1]. The bacteria culture-based methods, which are considered as the "gold standard" for the identification of bacterial pathogens, take normally >72 h [2], and

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show overall low positive rate due to the difficulty in choosing a proper culture medium and the overuse of antibiotics [3]. Such a long period and low positive rate limit the clinical value of bacterial identification, and consequently the accurate early antimicrobial therapy.

MALDI-TOF MS has been regarded as a revolutionary approach for the identification of microorganisms, including Gram-positive, Gram-negative bacteria, non-fermentative bacteria and anaerobes [4]. Biological molecules, typically small proteins originating from cell surfaces, intracellular membranes and ribosomes, constitute the fingerprint mass spectrum of a bacterium [5–7]. Fingerprint mass spectra acquired from bacterial samples are usually compared to reference spectra of purified known strains for identification. The MALDI-TOF MS based bacterial identification utilizes fewer reagents, fewer steps and requires less prior information of analytes than methods such as PCR and biochemical tests [8]. To date, it has already been used in clinical laboratories for

diagnosis. Nevertheless, the method requires single colonies for analysis [9, 10], wherein bacterial culture is still needed before MS analysis. As a result, the overall bacterial identification is still limited by bacterial culture.

Enrichment methods have been developed for fast bacterial identification to shorten or avoid bacterial culture step. Magnetic nano- and microparticles are desirable materials in affinity-based analytical methods due to the features of good biocompatibility and fast separation. Zhu et al. used rabbit anti-*E. coli* O157:H7 antibody modified magnetic nanoparticles as the capture probe for enrichment and separation of *E. coli* O157:H7, and functionalized silica-encapsulated gold nanoparticles as surface-enhanced Raman spectroscopy nanoprobe for detection [11]. Zeng et al. described a fluorometric and PCR based detection of *E. coli* and *S. aureus* using lysozyme as a reporter and magnetic microbeads as a carrier to enrich target bacterial DNA [12]. Xiong et al. developed a chemiluminescent method for the detection of *S. aureus*, wherein *S. aureus* competed with *Staphylococcus* protein A-modified magnetic beads for the binding to horseradish peroxidase-labeled IgG [13]. These methods mainly focus on detection of several specific species and show high sensitivity.

Magnetic particles based bacterial enrichment has also been used for sample pretreatment before MALDI-TOF MS analysis. Voorhees and co-workers were the first to employ immunomagnetic nanoparticles for selectively extracting bacteria in river water, human urine and blood from poultry meat for MALDI-TOF MS characterization. They achieved limit of detection (LOD) of 10^7 CFU·mL^{-1} bacteria in buffered aqueous solution [14]. Chen and coworkers have developed Fe_3O_4 magnetic nanoparticles modified with vancomycin to capture bacteria in human urine with LOD down to ~ 7.4×10^4 CFU·mL^{-1} [15]. They have also used pigeon ovalbumin to enrich *Escherichia coli* at the concentration of ~9.6×10^4 CFU·mL^{-1} in Tris buffer for MALDI-TOF MS analysis [16]. Zhu et al. used magnetic beads (MBs) modified with specific antibodies to capture target bacteria at concentrations as low as 500 CFU·mL^{-1} in serum or 8000 CFU·mL^{-1} in whole blood for MALDI-TOF MS identification [17]. Comparing to the other detection methods, MS can obtain bacterial fingerprint spectra as a direct proof of the presence of a bacterium to avoid false positive results and to achieve the identification of a wide range of species.

We establish a method using human IgG modified Fe_3O_4 MBs (IgG@Fe_3O_4) to enrich bacteria from human whole blood, followed by direct MALDI-TOF MS identification. IgG@Fe_3O_4 can bind to protein A [18], protein G [19], protein L [20] and glycans [21, 22] on the surface of bacterial cells to recognize a variety of species of bacteria. The performance of the method was demonstrated with six species of pathogenic bacteria, including Gram-positive *Staphylococcus aureus* and *Kocuria rosea* and Gram-negative *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*. Bacteria with concentrations as low as 10 CFU·mL^{-1} in water was efficiently extracted by the IgG@Fe_3O_4 with recovery rates >50%. Together with MALDI-TOF MS, the LOD of the tested strains was down to 10^3 CFU·mL^{-1} in water and 10^5 CFU in 100 μL human whole blood. To demonstrate its potential application in blood stream infections (BSIs) diagnosis, human whole blood samples with originally 10^2 CFU·mL^{-1} bacteria, which is the up-limit concentration of bacteria in the blood of BSIs patients [23], were prepared for blood culture. Bacterial identification from the blood samples was realized after short blood culture (BC) time, i.e. 4 to 6 h, when using the IgG@Fe_3O_4 in combination with MALDI-TOF MS. The whole enrichment and identification procedure took less than one hour. Thus, the method shows a promising value in rapid (i.e. in 8 h) clinical diagnosis of blood stream bacterial infections.

Experimental section

Chemicals and materials

Carboxyl MBs (HOOC-MBs, 10 mg·mL^{-1}, 200 nm in diameter) were purchased from Nanjing Nanoeast Biotech Co., Ltd. (Nanjing, China, www.nanoeast.net). Human whole blood was from Changhai Hospital (Shanghai, China). Human IgG (≥95%), bovine serum albumin (BSA), 4-morpholineethanesulfonic acid (MES) (≥99%), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) (≥98.0%), N-hydroxysuccinimide (NHS) (98%), 2,5-dihydroxy-benzonic acid (DHB) (>99.0%), tween-20 and RIPA lysis buffer were purchased from Sigma-Aldrich Inc. (Saint Louis, USA, www.sigmaaldrich.com). Acetonitrile (≥99.9%) was purchased from Merck (Darmstadt, Germany, www.merck.com). Trifluoroacetic acid (TFA) (99.0%) was from Adamas Reagent Co., Ltd. (Shanghai, China, www.adamas-beta.com). Phosphate buffered saline (PBS: 136.89 mmol·L^{-1} NaCl, 2.67 mmol·L^{-1} KCl, 8.24 mmol·L^{-1} Na$_2$HPO$_4$, 1.76 mmol·L^{-1} KH$_2$PO$_4$ in DI water, pH 7.4) was from Sangon Biotech Co., Ltd. (Shanghai, China, www.sangon.com). Trypticase soybroth (TSB), Luria-Bertani (LB) medium, nutrient broth (NB) and tryptone soya agar (TSA) were from Beijing Land Bridge Technology Co., Ltd. (Beijing, China, www.beijinglandbridge.com). Blood culture bottles were from Autobio Diagnostics Co., Ltd. (Zhengzhou, China, www.autobio.com.cn). Deionized (DI) water (18.2 MΩ cm) was purified by a Smart-Q deionized water system (Hitech pure water technology, Shanghai, China, www.high-tech.cn) and used in all aqueous solutions.

Culture of bacteria

Klebsiella pneumoniae (*K. pneumoniae* strain ATCC 700603), *Staphylococcus aureus* (*S. aureus* strain ATCC 25923), *Klebsiella oxytoca* (*K. oxytoca* strain ATCC 27853), and *Pseudomonas aeruginosa* (*P. aeruginosa* strain ATCC 27853) were cultured in 20 mL TSB medium at 37 °C for 14 h with consecutive shaking at 175 rpm. *Kocuria roseus* (*K. roseus* strain ATCC 9815) were cultured in 20 mL NB medium at 26 °C for 14 h with consecutive shaking at 175 rpm. *Enterobacter cloacae subspecies dissolvens* (*E. cloacae sub. Dissolvens* strain ATCC 23373) were cultured in 20 mL NB medium at 28 °C for 14 h with consecutive shaking at 175 rpm. The rough concentrations of bacteria in the broths were derived by measuring the absorbance at the wavelength of 600 nm using APL 759 UV-visible absorption spectroscopy (Shanghai, China). The accurate concentrations of bacteria were determined by plate counting.

Extraction of bacteria from aqueous samples by IgG@Fe$_3$O$_4$

IgG was modified on Fe$_3$O$_4$ MBs to form IgG@Fe$_3$O$_4$ via EDC-NHS reaction [24] and the detailed protocol for the modification is in Electronic Supporting Material. The prepared IgG@Fe$_3$O$_4$ were stored in PBST (0.05% Tween-20 in PBS) at 4 °C for use. A series of bacteria aqueous suspension (10^6, 10^5, 10^4, 10^3 CFU·mL^{-1}) were prepared. 1.5 μg IgG@Fe$_3$O$_4$ were added to 1 mL bacteria suspension to capture the bacteria. After incubation at 37 °C for 30 min with consecutive shaking, the bacteria@IgG@Fe$_3$O$_4$ conjugates were collected and washed once with 200 μL PBST (saline/Tween) followed by washing once with 200 μL DI water. The bacteria@IgG@Fe$_3$O$_4$ conjugates were then deposited on a Bruker Anchorchip™ target plate (Bremen, Germany) for subsequent MALDI-TOF MS analysis.

Extraction of bacteria from human blood

A series of human blood samples spiked with bacteria (10^7, 10^6, 10^5, 10^4 CFU in 100 μL human whole blood) were prepared. The blood samples were collected from healthy volunteers in Changhai Hospital (Shanghai, China). 1 mL DI water was added to the 100 μL blood sample, and incubated for 5 min at 37 °C. The mixture was centrifuged at 3000 *g* for 10 min to discard the supernatant. Then, 0.5 mL RIPA lysis buffer was added to the obtained pellet, incubated for 5 min at 37 °C. The mixture was centrifuged at 3000 *g* for 10 min to discard the supernatant. The pellet was washed twice with 1 mL DI water. The collected pellet was then resuspended in 100 μL PBST. 3 μg IgG@Fe$_3$O$_4$ were added to the mixture to capture the bacterial cells. The procedures of bacterial extraction and MALDI-TOF MS detection were same as the ones used for the aqueous samples.

Extraction of bacteria from BC samples

5 mL whole blood of a healthy volunteer was spiked with *K. pneumonia* or *S. aureus* to reach the final concentration of 100 CFU·mL^{-1}, and then injected into a BC bottle and cultured at 37 °C with consecutive shaking. The BC bottles contained various growth factors, amino acids and vitamins to promote the rapid reproduction of common bacteria. For each species, two BC bottles were prepared in parallel. During the process of BC, 1 mL culture liquid was collected from one of the parallel bottles every 2 h (0 h, 2 h, 4 h, 6 h, 8 h and 10 h) and analyzed with the IgG@Fe$_3$O$_4$ based extraction and MALDI-TOF MS detection, while the other bottle was left untouched until turned positive, i.e. to reach ~10^8 CFU·mL^{-1} bacteria in the bottle. For IgG@Fe$_3$O$_4$ based extraction, 1 mL BC sample was centrifuged at 140 *g* for 10 min to remove erythrocytes. The supernatant was collected and centrifuged at 16,000 *g* for 3 min. The sediment was washed twice with 1 mL DI water to obtain bacterial pellets. Finally, the pellet was re-suspended in 100 μL PBST. 3 μg IgG@Fe$_3$O$_4$ were added to the solution to capture bacterial cells. The procedures of bacterial extraction and MALDI-TOF MS detection were same as the one used for the aqueous samples.

MALDI-TOF MS detection of cultured bacteria

1 μL sample suspension was deposited on a sample spot of a MALDI target plate with four repetitions (0.25 μL per repetition) droplet-by-droplet [17]. DHB matrix (1 μL, 10 mg·mL^{-1} in $V_{acetonitrile}/V_{water}/V_{TFA}$ 50/49.9/0.1) was deposited to overlay the dried sample spots also with the droplet-by-droplet protocol for MALDI-TOF MS analysis. All MALDI-TOF MS detections were performed on a Bruker MicroFlex LRF mass spectrometer (Bremen, Germany) under linear positive mode. The instrumental parameters were set as: 75% laser intensity, laser attenuator with 35% offset and 40% range, accumulation from 400 laser shots, 10.3× detector gain, and 150 ns delayed extraction time. Aqueous solution containing cytochrome c, myoglobin, insulin and ubiquitin was used for external mass calibration. For each enrichment experiment, there were at least triplicate samples in parallel, and the results shown in the paper were randomly chosen from all the obtained mass spectra.

Results and discussion

Choice of materials

Due to the magnetic properties, magnetic beads (MBs) are often used as the substrate of affinity probes for selective enrichment of target species. We have compared MBs with

different size, i.e. 10 nm, 200 nm and 1 μm. 10 nm MBs cannot provide sufficient magnetic force when using a normal commercialized magnetic strand for separation. The size of 1 μm MBs is similar to the size of bacteria, thus the contact cross section between 1 μm MBs and bacteria is small, and the extraction efficiency was not good. Among the three different sizes, 200 nm MBs showed best performance in extracting bacterial for MALDI-TOF MS characterization. HOOC-MBs were chosen instead of protein A/G coated MBs for the modification of IgG via EDC-NHS reaction. Although the protein A/G coated MBs can be easily modified by IgG, it will occupy the Fc domain of IgG, which is also needed during the affinity interaction between IgG and bacterial cells. Therefore, carboxyl MBs with the size of 200 nm were finally chosen for the enrichment of bacteria for MALDI-TOF MS characterization.

Extraction of bacteria by IgG@Fe$_3$O$_4$

In a previous work, an immunoaffinity based bacterial extraction method has been developed, wherein specific antibodies were used to modify MBs to capture corresponding bacterial strains for subsequent MS analysis [17]. We aim at bacteria enrichment in a non-targeted way. For this purpose, IgG@Fe$_3$O$_4$ were prepared. Human IgG is a target protein of many pathogenic bacteria. The interaction between IgG and protein A on the cell membranes of *S. aureus* was discovered almost 50 years ago [18]. Protein A can bind to the Fc region of IgG in a process usually called pseudo-immune reaction, different from the specific binding of antigens to antibodies at the Fab region [25]. The IgG@Fe$_3$O$_4$ was used to extract various bacterial species from water or human blood for subsequent MALDI-TOF MS identification. The assay is schematically illustrated in Scheme 1. The key steps in developing this

assay include the modification of IgG to Fe$_3$O$_4$ MBs, the specific binding of the IgG@Fe$_3$O$_4$ to bacterial cells, and sensitive detection of bacteria@IgG@Fe$_3$O$_4$ using MALDI-TOF MS. Each of the steps determines the sensitivity and specificity of the assay.

Pure Fe$_3$O$_4$ MBs and IgG@Fe$_3$O$_4$ were characterized by scanning electron microscopy (SEM), respectively. It was observed that the particle size hardly changed before and after modification (ESM Fig. S1), while the modification can be visually observed from the images. In addition, the IgG@Fe$_3$O$_4$ beads were analyzed by MALDI-TOF MS to confirm the successful modification of Fe$_3$O$_4$ MBs with IgG (ESM Fig. S2).

Affinity of the IgG@Fe$_3$O$_4$ towards bacteria was demonstrated by fluorescence microscopy and SEM, using *S. aureus* as a model sample. 50 μL 100 μg·mL^{-1} fluorescein isothiocyanate isomer I (FITC)-tagged human IgG was incubated with 100 μL 10^8 CFU·mL^{-1} *S. aureus* aqueous suspension at 37 °C for 30 min. After incubation, the bacterial cells were collected and washed three times with 1 mL DI water, and then observed by a fluorescence microscopy with a magnification of ×1000. The bacteria were observed in bright and dark fields, indicating that IgG indeed bound well to *S. aureus*, as shown in Fig. 1(a) and (b). Affinity of the IgG@Fe$_3$O$_4$ to bacteria was also directly observed by SEM, as shown in Fig. 1(c), (d) and (e).

Bacterial extraction efficiency by the IgG@Fe$_3$O$_4$ was determined using plate count method. 1.5 μg IgG@Fe$_3$O$_4$ were added into 1 mL bacterial suspension at various concentrations, and incubated for 30 min. Bacteria in original aqueous sample, captured by IgG@Fe$_3$O$_4$, and left in supernatant were quantified. It was found that the ratios of captured bacteria were always larger than 50% with different concentrations (10 to 10^3 CFU·mL^{-1}) of bacteria, as shown in Fig. 2.

Scheme 1 (a) Modification of Fe$_3$O$_4$ MBs with IgG via EDC-NHS reaction; (b) schematic illustration of bacteria extraction by IgG@Fe$_3$O$_4$ from sample solution for MALDI-TOF MS identification
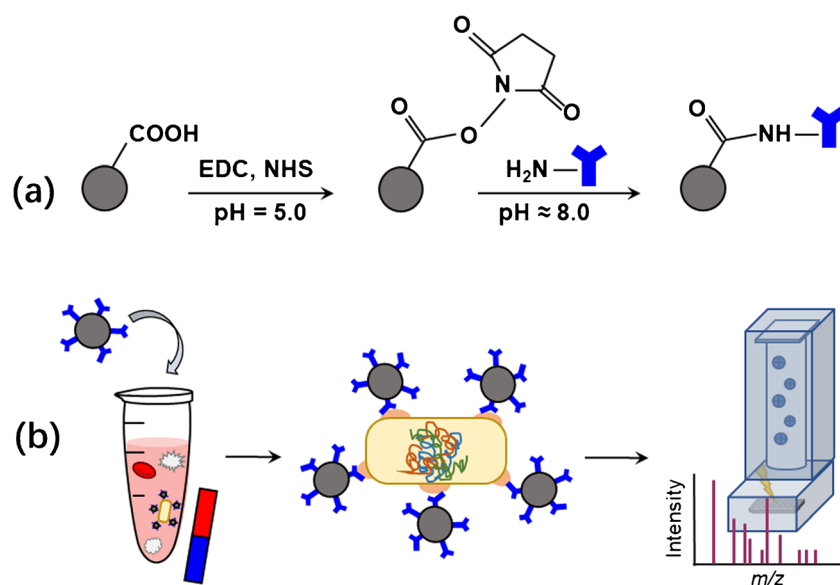
Fig. 1 Fluorescence microscopy images of *S. aureus* conjugated to FITC-labeled IgG: (**a**) bright field image and (**b**) green fluorescence image. SEM images of (**c**) IgG@Fe$_3$O$_4$, (**d**) a *S. aureus* cell, and (**e**) the *S. aureus* conjugated IgG@Fe$_3$O$_4$. The scale bars in (**c**), (**d**) and (**e**) indicate 500 nm. Fluorescence microscopy was recorded by an Olympus BX53-DP80 (Tokyo, Japan) microscope. SEM was recorded by a Hitachi S-4800 (Tokyo, Japan) microscope
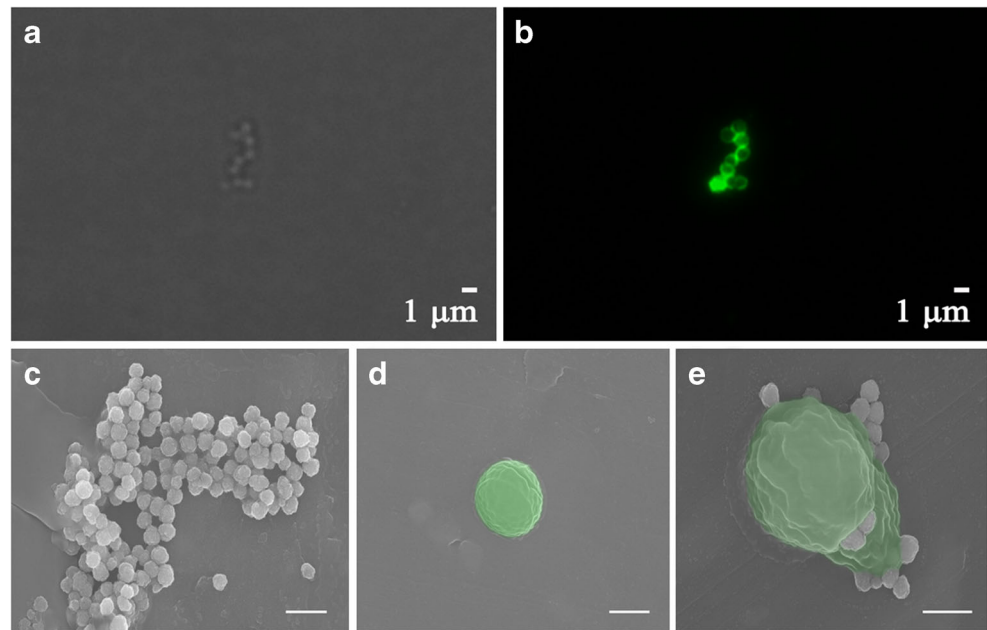

Detection of bacteria from water

Bacteria extracted by IgG@Fe$_3$O$_4$ can be analyzed by MALDI-TOF MS. For sensitive detection, incubation time and amount of IgG@Fe$_3$O$_4$ were optimized. Respective data and figures are given in the ESM (Figs. S3 and S4). The following experimental conditions were found to give best results: (a) optimal incubation time: 30 min, and (b) optimal amount of IgG@Fe$_3$O$_4$: 1 to 3 μg.

Under the optimized condition, different species (*S. aureus, K. rosea, K. pneumoniae, K. oxytoca, E. cloacae* and *P. aeruginosa*) in water with various concentrations were enriched by the IgG@Fe$_3$O$_4$ and analyzed by MALDI-TOF MS. As shown in Fig. 3, good MS signal was obtained when

Fig. 2 The photograph of spread plate of original solution, *S. aureus*@IgG@Fe$_3$O$_4$ and supernatant. The efficiency was calculated from three replications
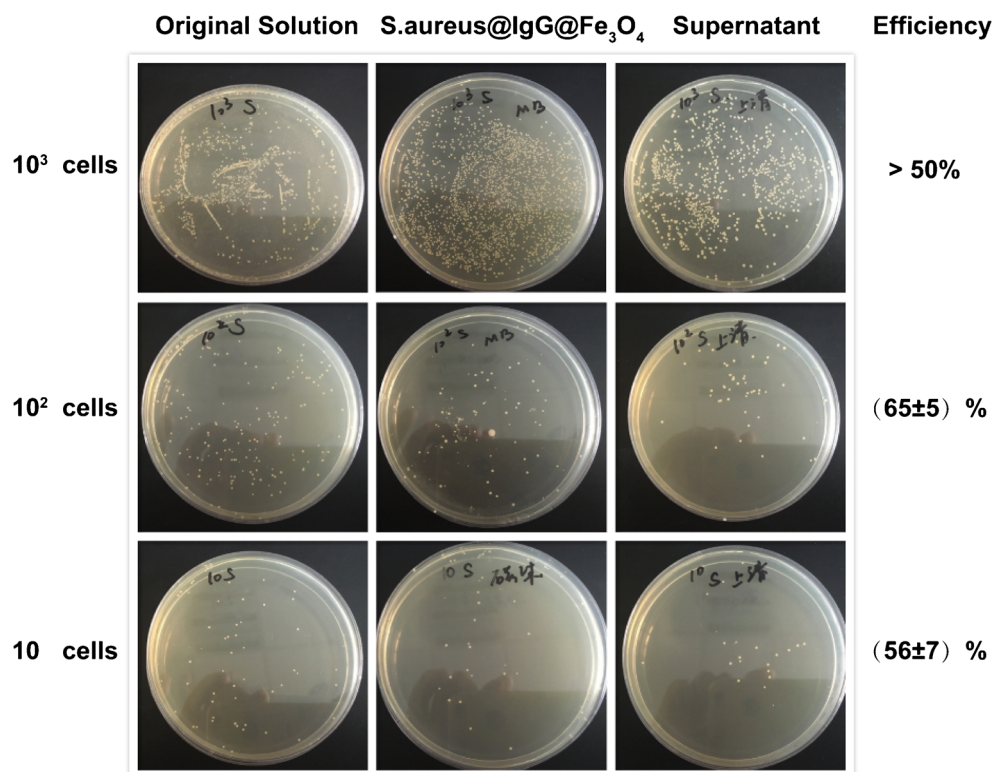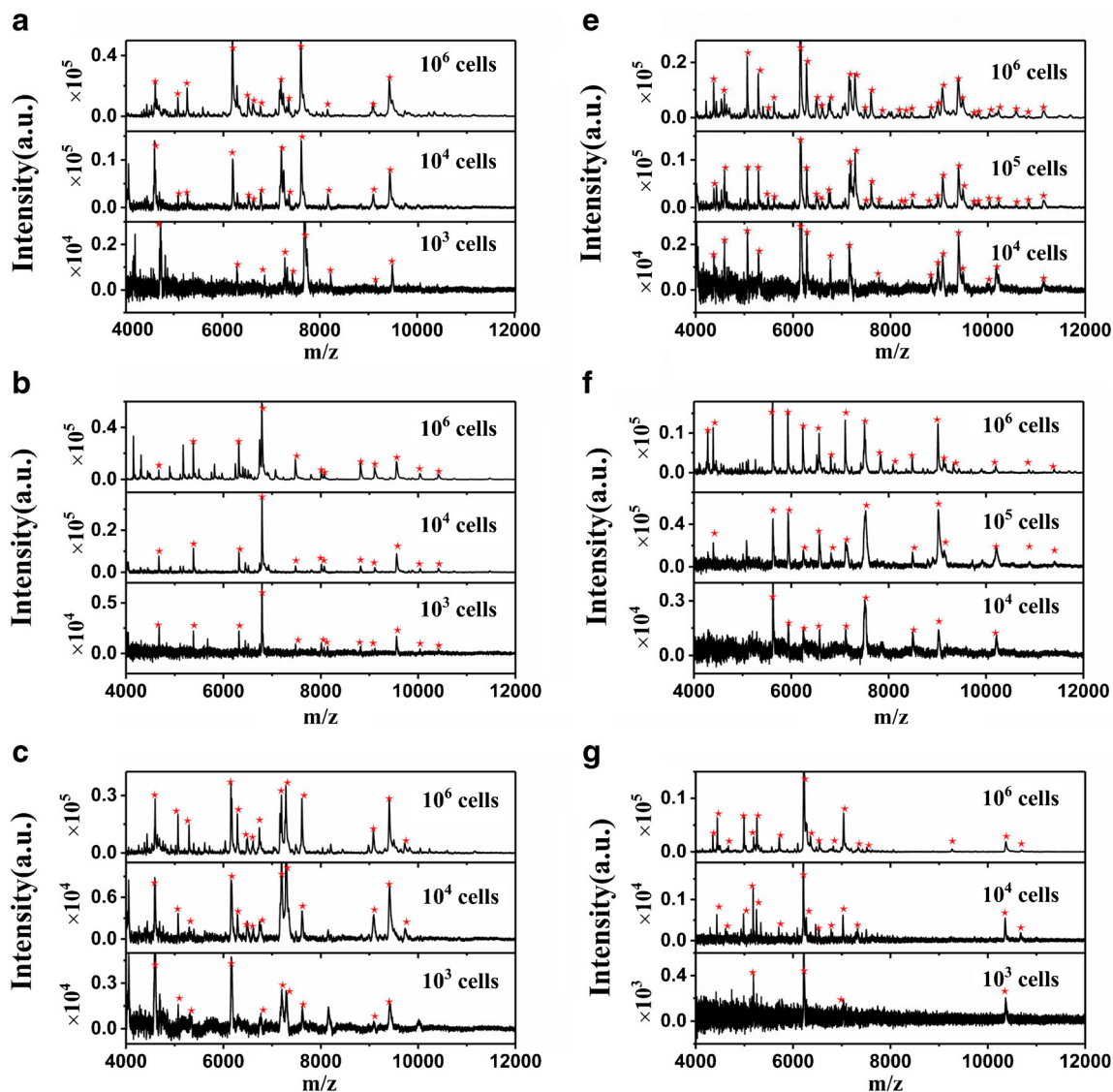
Fig. 3 MALDI-TOF mass spectra of (**a**) *K. pneumoniae*, (**b**) *S. aureus*, (**c**) *K. oxytoca*, (**d**) *E. cloacae sub. Dissolvens*, (**e**) *P. aeruginosa* and (**f**) *K. roseus* aqueous samples (1 mL) at different amounts as indicated on the figures after enrichment by the IgG@Fe$_3$O$_4$. Peaks corresponding to the bacterial cellular components are denoted by asterisks

the concentration of bacteria in water was as low as 10^3 CFU·mL^{-1}. Peaks marked on Fig. 3 represent cellular components of the corresponding bacteria, well in agreement with the MS results of pure cultures (ESM Fig. S5). Identification of bacteria can be realized based on the observation of peaks specific to the bacteria with S/N > 3.

Without using the bacterial extraction method, bacterial suspension with the concentrations of >10^7 CFU·mL^{-1} is usually required for efficient MALDI-TOF MS based identification [10], wherein 1 μL of sample solution is directly deposited on a MALDI sample spot for MS analysis. Combing the IgG@Fe$_3$O$_4$ extraction and MALDI-TOF MS analysis, especially using the droplet-by-droplet sample preparation method or the Anchorchip™ target plate, bacteria were identified from

1 mL 10^3 CFU·mL^{-1} aqueous solution. With the strategy, the period for bacteria culture before MADLI-TOF MS analysis can be largely shortened or even avoided to fasten bacterial identification.

Detection of bacteria from human blood

Clinical samples used for bacterial identification during clinical diagnosis include blood, urine, cerebrospinal fluid, prostatic fluid, *etc* [26]. Among the samples, bacteria in blood are most difficult to be detected due to the complex sample matrices, e.g. highly abundant blood proteins, red blood cells, leucocytes and platelets [27]. Bacteria culture is usually
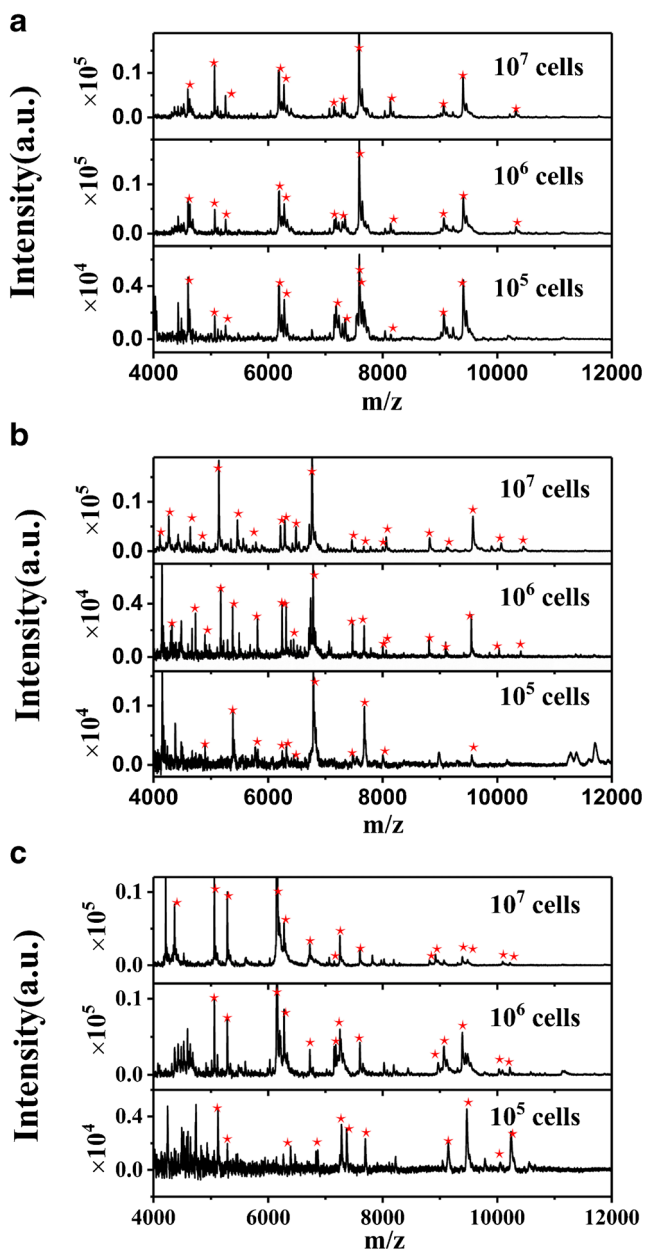
Fig. 4 MALDI-TOF mass spectra of (**a**) *K. pneumoniae*, (**b**) *S. aureus*, and (**c**) *K. oxytoca* in 100 μL human whole blood samples at different amounts as indicated on the figures after enrichment by IgG@Fe$_3$O$_4$. Peaks corresponding to bacterial cellular components are denoted by asterisks

required to purify bacterial cells and to amplify the number of cell copies for the diagnosis of BSIs.

To demonstrate the value of the method for clinical usage, human whole blood samples were spiked with standard strains with known concentrations to mimic clinical samples of BSIs patients. The IgG@Fe$_3$O$_4$ based bacterial extraction protocol was combined with simple sample pretreatment methods to detect bacteria directly from the spiked human whole blood, as detailed in the experimental section. In order to minimize non-specific adsorption of IgG@Fe$_3$O$_4$ towards the highly

abundant proteins in human blood, a blocking buffer (1% BSA dissolved in PBST) was used during the experiments. Human whole blood spiked with *K. pneumoniae*, *S. aureus* and *K. oxytoca* were analyzed by the method. As shown in Fig. 4, 10^5 bacterial cells in 100 μL human whole blood sample can be detected by the IgG@Fe$_3$O$_4$ in coupling with MALDI-TOF MS.

The bacterial identification from human blood samples was not as efficient as that from the aqueous samples. The reason can be ascribed to the loss of bacteria during the sample pretreatment processes, i.e. cell lysis, centrifugation and washing steps, and the non-specific affinity of IgG to serum proteins and blood cells. Nevertheless, the value of the IgG@Fe$_3$O$_4$ based bacterial extraction is clear when comparing to the results without affinity capture, as shown in ESM Fig. S6, where human blood samples were only treated with eukaryocytes lysis, centrifugation and washing to obtain bacterial cell pellets. Without the IgG@Fe$_3$O$_4$ based bacterial extraction, many peaks from serum proteins were observed (Fig. S6), inhibiting the observation of bacterial signals.

The sensitivity of the method does not match the concentrations of bacteria in clinical blood samples, which are normally ≤10^2 CFU·mL^{-1} [23]. Nevertheless, it is possible to couple the method with BC for bacterial identification. The time required for BC can be largely shortened when using the IgG@Fe$_3$O$_4$ based bacterial extraction for sample preparation. As shown in Fig. 5, a test with fixed time interval was implemented to investigate the influence of BC time on identification result. BC started with human blood samples containing 10^2 CFU· mL^{-1} *S. aureus* or 10^2 CFU·mL^{-1} *K. pneumonia*. After 6 h of BC, mass spectra with good quality were obtained from 1 mL BC solution using the IgG@Fe$_3$O$_4$ combined MALDI-TOF MS analysis while only after ~10 h of BC the bottles turned positive. These results indicate that this method enables identification of bacteria before BC bottles turn positive.

A comparison of the current approach and relevant methods based on MALDI-TOF MS for the analysis of pathogenic bacteria is listed in Table 1. Target bacteria, sample type, incubation time, and LOD are involved in the comparison. Our work adopted more species and demonstrated the potential application of non-targeted pathogenic bacterial identification from human whole blood. There were three kinds of samples involved in our work: bacterial aqueous solution, bacteria in human blood and blood culture samples. Comparing to the work of Zhu et al. [17], our work is not limited by the avaibility of specific antibodies, and needs less prior information of analytes. From a broader perspective, our method can also be used as an adjuvant tool in therapy in order to assist in the treatment of sepsis through extraction of pathogenic bacteria from patients' blood.

We have noticed that the IgG@Fe$_3$O$_4$ showed varied affinity to different species. Within the six species, it was more difficult to identify *E. cloacae sub. Dissolvens*, *P. aeruginosa* and
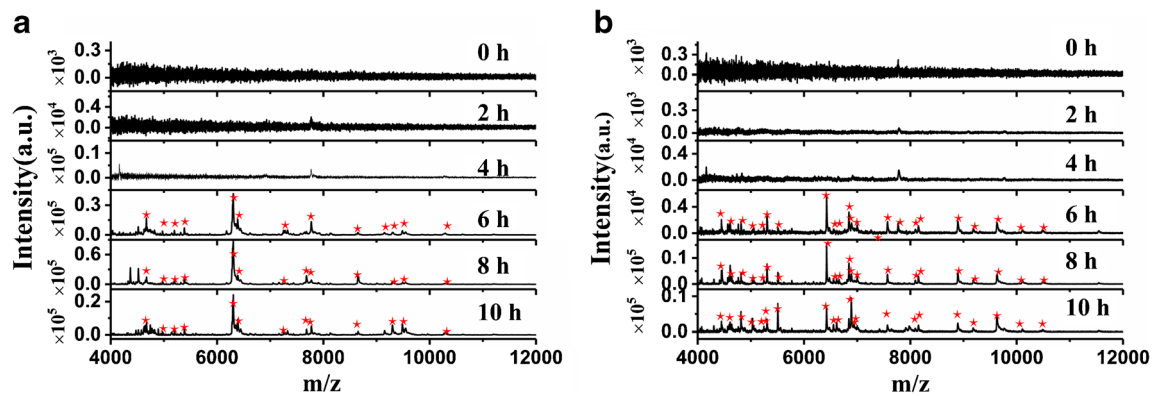
Fig. 5 MALDI-TOF mass spectra obtained from BC samples: (**a**) *K. pneumoniae* and (**b**) *S. aureus* with initial concentrations of 10^2 CFU·mL^{-1} in 5 mL human whole blood after different BC times: 0 h, 2 h, 4 h, 6 h, 8 h and 10 h, and enrichment by IgG@Fe$_3$O$_4$. Peaks corresponding to bacterial cellular components are denoted by asterisks

K. roseus as shown on Fig. 3, where higher concentrations of the bacteria were needed to generate mass spectra at the similar quality compared to *K. pneumoniae*, *S. aureus*, and *K. oxytoca*. Performance to other bacterial cells needs to be further investigated. The IgG@Fe$_3$O$_4$ based method, especially when considering complex clinical samples (human blood), is less sensitive compared to the method of Zhu et al. [17] wherein specific antiodies were used. The equilibrium dissociation constant (K$_D$) between antigen and corresponding specific antibody can be down to pM [30, 31], which in many cases is smaller than the K$_D$ of interaction between IgG and protein A (0.14 μM) [32]. When the sample is blood, IgG can also bind to serum proteins and blood cells via unspecific interaction, which can further hinder the sensitivity of the method. These are the main limitations of the method to be considered for real applications in clinical diagnosis.

Conclusion

A non-targeted and fast assay for bacterial identification from human blood based on the enrichment of bacteria by IgG modified MBs and identification by MALDI-TOF MS has been demonstrated. Since the enrichment is based on the affinity between IgG and the bounding sites, such as protein A, protein G, protein L, glycans etc., on the surface of bacteria

Table 1 Comparison of the current approaches for the analysis of pathogenic bacteria using functionalized magnetic particles coupled MALDI-TOF MS

Functional groups modified on magnetic particles	Tested bacteria	Sample type	Sample volume (mL)	Incubation time (min)	LOD (CFU·mL^{-1})	Reference
Vancomycin-bound magnetic nanoparticles	*Staphylococcus saprophyticus* *Staphylococcus aureus*	Urine	3	60	7.4×10^4 7.8×10^4	[15]
Pigeon ovalbumin-bound Fe$_3$O$_4$@Al$_2$O$_3$ magnetic nanoparticles	P fimbriated *Escherichia coli*	Tris buffer	0.5	60	9.6×10^4	[16]
Vancomycin-conjugated magnetic nanoparticles	*Bacillus cereus*, *Enterococcus faecium*, *Staphylococcus aureus*	Water	2000	30	5×10^2	[28]
Anti- *Escherichia coli* O157-modified MBs	*Escherichia coli* O157:H7	Meat extract bacterial suspension	1	20	2×10^6	[29]
Specific antibody-modified MBs	*Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*	Blood serum Whole blood	1	30	500 8000	[17]
IgG-modified magnetic particles	*Staphylococcus aureus*, *Kocuria rosea*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*	Water Human whole blood	1	30	10^3 10^6	This work

that exist in many different species, we expect that the current strategy is not limited to the tested six species. Comparing to traditional BC method, the analysis time is largely shortened and can be finished in 8 h. The sensitivity of the method needs to be further enhanced, possibly by the development of new affinity probes, new matrix for MALDI-TOF MS, and MS detector. In addition, the IgG@Fe$_3$O$_4$ is not limited to the combination with MALDI-TOF MS, it can also be combined with many other identification methods, e.g. PCR, Raman spectroscopy, infrared spectroscopy, etc.

Compliance with ethical standards The author(s) declare that they have no competing interests.

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