Biosensors and Bioelectronics 54 (2014) 378–384

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Microconductometric immunosensor for label-free and sensitive detection of Gram-negative bacteria

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ARTICLE INFO

Article history:
Received 9 August 2013
Received in revised form
18 October 2013
Accepted 5 November 2013
Available online 14 November 2013

Keywords:
Blood safety
Gram-negative bacteria
Magneto-immunoassay
Conductometric transduction
Label free detection

ABSTRACT

Blood safety is a global health goal. In developed countries, bacterial contamination of platelet concentrates is the highest infectious risk in transfusion despite the current preventive strategies. We aimed to develop a conductometric biosensor for the generic, rapid and sensitive detection of Gram-negative bacteria. Our strategy is based on immunosensors: addressable magnetic nanoparticles coupled with anti-LPS antibodies were used for the generic capture of Gram-negative bacteria. Bacterial capture was characterized by impedancemetric and microscopic measurements. The results obtained with conductometric measurements allowed real-time, sensitive detection of *Escherichia coli* or *Serratia marcescens* cultures from 1 to 10^3 CFU mL^{-1}. The ability of the immunosensor to detect Gram negative bacteria was also tested on clinically relevant strains. The conductometric immunosensor allowed the direct detection of 10–10^3 CFU mL^{-1} of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains that were undetectable using standard immunoblot methods. Results showed that the conductometric response was not inhibited in 1% serum.

1. Introduction

Blood testing must be able to adapt to the demands of microbiological safety since the offensive engaged against blood-transmitted agents remains ongoing. Bacterial contamination of blood components is the highest infectious risk in transfusion despite the current preventive strategies (Vollmer et al., 2012, Murphy and Coakley, 2011). If all the precautionary measures are taken, the number of contaminating bacteria is initially very small and it is estimated that a blood donation contains 10–100 bacteria (i.e. 0.03–0.3 CFU mL^{-1} if referring to a platelet concentrate having a mean volume of 300 ml). While bacterial contamination may affect any blood component, the ambient storage temperature conditions for platelets make them the most likely to facilitate bacterial growth (Palavecino et al., 2010). Although Gram-positive bacteria are commonly recovered from contaminated platelets, Gram-negative bacteria are more likely to result in clinically severe reactions and fatalities (Palavecino et al., 2010).

The various approaches developed to date to detect the presence of bacteria in labile blood products are long, expensive, and still present many limitations according to recent reviews (Palavecino et al., 2010; Schmidt et al., 2011; Schmidt and Seifried, 2012). Many countries have implemented bacterial culture methods but false negative screening results have been reported in some cases, mainly due to early sampling errors. Thus, the test sample is performed on the blood product after an incubation period (usually 24 h) but may be inefficient for detecting bacteria with slow growth in the blood product (Murphy and Coakley, 2011). Finally, many countries have implemented bacterial culture methods with the negative-to-date concept (Table 1). Alternative strategies are needed to address the problem of bacterial contamination in platelets. Within the last few years, a couple of rapid bacterial detection methods have been developed including: flow cytometry-based methods, impedance measurements, immunoassays or bacterial genome amplifications (Palavecino et al., 2010; Vollmer et al., 2010, 2011, 2012; Mueller et al., 2012; Rood et al., 2011; Rieder et al., 2011; Patel et al., 2012) (Table 1). We are far from an ideal bacterial screening system (Palavecino et al., 2010; Schmidt and Seifried, 2012). The rapid control of blood components carried out near the time of issue for transfusion could represent an innovative safety approach.

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http://dx.doi.org/10.1016/j.bios.2013.11.016

Table 1
Characteristics of relevant bacterial detection methods, including electrochemical biosensors, reported in the literature.

Detection method	Immobilization technique	Application	Bacteria	Detection limit (CFU mL^{-1})	Working range (CFU mL^{-1})	Detection time	References
Validated conventional methods							
Culture methods (CO_2 analysis)	–	Clinical	All bacteria	10	–	Negative to date concept	Palavecino et al. (2010), Schmidt and Seifried (2012)
Culture methods, (O_2 analysis)	–	Clinical	Aerobic and facultative anaerobic bacteria	<50	–	24 h	Fournier-Wirth et al. (2006), Palavecino et al. (2010), Schmidt and Seifried (2012)
O_2 sensor	–	Clinical	All bacteria	4×10^2	4×10^2–10^5	25.3 h	Mueller et al. (2012)
Flow cytometry	–	Clinical	All bacteria	1.5×10^2	–	60 min	Vollmer et al. (2011)
Lateral flow, immunoassays	–	Clinical	All bacteria	10^4	10^4–10^6	30–90 min	Vollmer et al. (2010)
Peptidoglycan test	–	Clinical	All bacteria	10^4	–	60 min	Palavecino et al. (2010)
Real time PCR	–	Clinical	All bacteria	1	1×10^5	4 h	Patel et al. (2012)
Innovative electrochemical techniques							
Amperometry	Antibody-coated gold nanoparticles	Food	*E. coli*	10^2	10^2–10^7	60 min	Lin et al. (2008)
	Antibody-coated paramagnetic particles	Environment	*E. coli*	2×10	$2\times10-10^6$	7 h	Boyaci et al. (2005)
	Antibody-coated magnetic particles	Food/clinical	*E. coli*	10^2	10^2–10^8	60 min	Laczka et al. (2011)
Voltammetry	Physical adsorption of antibodies onto polyaniline surface	Food	*E. coli*	7×10	7×10–10^4	70 min	Setterington and Alocilja (2011)
	Antibody-coated magnetic particles	Food	*S. enteretica*	10^3	10^3–10^6	90 min	Afonso et al. (2013)
	DNA detection	Clinical	*E. coli*	4.8×10	4.8×10–10^5	35 min	Safavieh et al. (2012)
Impedance	Antibody-coated magnetic nanoparticles	Food	*E. coli*	8×10^5	8×10^5–10^7	35 min	Varshney and Li (2007)
	Biotinylated polyclonal anti-*E. coli*- coated SAM	Clinical	*E. coli*	10	10–10^4	60 min	Maalouf et al. (2007, 2008)
	Biotinylated polyclonal anti-*E. coli*- coated SAM	Food/clinical/ environment	*E. coli*	2	10–10^4	2 min	Barreiros dos Santos et al. (2013)
	Differential impedance platform	Clinical	Stressed bacteria	10^3	10^3–10^6	30 min	Rieder et al. (2011)
Conductometry	Polyclonal antibody immobilization on a nitrocellulose membrane	Food	*E. coli*	8×10	$8-10$–10^4	2–10 min	Muhhammad-Tahir and Alocilja (2003)
	Antibody-coated magnetic nanoparticles	Clinical	*E. coli*	1	1–10^3	2 min	This work
			S. marcescens	1	1–10^3	2 min	
			A. baumannii	10	10–10^3	2 min	
			P. aeruginosa	10	10–10^3	2 min	

The development of biosensing nanotechnologies may allow blood testing to switch to new miniaturized platforms (Fournier-Wirth and Coste, 2010; Fournier-Wirth et al., 2010).

Progress in nanostructured and nanoscale materials has offered new alternatives for the detection of bacteria thanks to the fabrication of rapid biosensors with improved sensitivity as reviewed recently (Heo and Hua, 2009; Nayak et al., 2009; Velusamy et al., 2010; Vikesland and Wigginton, 2010; Arora et al., 2011; Ceylan Koydemir et al., 2011; Sapsford et al., 2011; Wang et al., 2012a, 2012b). Electrochemical sensors have a strong potential to be used as innovative assays for the direct detection of bacterial contaminants (Muhhammad-Tahir and Alocilja, 2003; Varshney and Li, 2007; Lin et al., 2008; Laczka et al., 2011; Setterington and Alocilja, 2011; Safavieh et al., 2012; Afonso et al., 2013) (Table 1). Among these devices, the advantages of conductometric transducers have been extensively discussed (Jaffrezic-Renault and Dzyadevych, 2008).

Gram-negative and Gram-positive bacteria present lipopolysaccharides (LPS) and lipoteichoic acids (LTA) respectively as conserved antigens on their membrane (Caroff and Karibian, 2003; Sutcliffe and Shaw, 1991). Antibodies directed against LPS and LTA can be used as bioreceptor for the generic detection of bacteria. The antigen–antibody interaction allows changes in the electrical properties of the immobilized antibody layer on the electrode which can be monitored by electrochemical impedance measurements. There are many strategies and supports for the immobilization of antibodies. Magnetic particles are particularly interesting since they offer many advantages such as optimized target capture, signal amplification, easy manipulation with no need for chemical reagents which can alter immobilized proteins, stability over time, possibility of integration in microfluidic devices, etc. (Jaffrezic-Renault et al., 2007; Tamanaha et al., 2008; Laczka et al., 2011). In a previous work, we designed an immunomagnetic sensor using a magnetic field to attach a streptavidin functionalized paramagnetic nanobead layer onto the electrode surface. Impedimetric measurements allowed *E. coli* detection at concentrations ranging from 10 to 10^4 CFU mL^{-1} (Maalouf et al., 2008). This impedimetric immunosensor needed to be improved as 1 h of incubation time was necessary before each measurement. The antigen–antibody interaction can also produce a change in conductance between a pair of electrodes. We evaluated the ability of a conductometric transducer based on interdigitated electrodes, to detect whole bacteria in another work using biotinylated antibody anti-*E. coli* grafted onto streptavidin modified magnetic nanoparticles (Hnaiein et al., 2008). The interdigitated electrodes allow the measurement of the change of conductivity in the region defined by field lines. The involved thickness is of the order of the interdigit distance (few tens of micrometre) (Pänke et al., 2008). In the high frequency range (more than 1 kHz), these interdigitated electrodes allow the measurement of the change of ionic conductivity, in the boundary layer (Sheppard et al., 1996). This point has been extensively applied for the conductometric detection of substrates of enzymatic reactions, through the change in the co-enzyme concentration or in the product concentration (Jaffrezic-Renault and Dzyadevych, 2008; Temple-Boyer et al., 2008).

In the present work, we have developed a conductometric immunosensor using interdigitated microelectrodes. Magnetic nanoparticles functionalized with anti-LPS antibodies were used for the generic capture of Gram-negative bacteria. Different types of Gram-negative bacteria were tested, including clinically relevant bacteria, using both the designed microconductometric immunosensor and standard immunoblot methods. Gram-positive bacteria (*S. epidermidis*) were tested as a negative control. This strategy could be of special interest to detect relevant Gram-negative bacteria in medical practices.

2. Experiments

2.1. Microorganism, biomaterials and chemicals

All bacteria strains, *Escherichia coli* (CIP 76.24), *Serratia marcescens* (CIP 103235), *Pseudomonas aeruginosa* (CIP 76.110), *Acinetobacter baumannii* (CIP 70.34) and *Staphylococcus epidermidis* (CIP 68.21), originated from the bacterial collection of the Pasteur Institute (Paris, France). Trypticase Soy Broth medium (TSB) and Mueller Hinton agar plates were purchased from Biomérieux (Craponne, France). Purified lipopolysaccharide (LPS) was purchased from Sigma-Aldrich (Saint Quentin Fallavier, France).

A mouse monoclonal anti-LPS antibody (IgG2b, 0.5 mg mL^{-1}) and a goat polyclonal anti-LPS antibody (IgG, 3.5 mg mL^{-1}) were obtained from Abcam Company (Cambridge, UK). Carboxylated super-paramagnetic particles of 200 nm diameter, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), activation buffer and storage buffer were purchased from Ademtech (Pessac, France).

Bovine serum albumin (BSA), glutaraldehyde (GA) (grade II, 25% aqueous solution), MES (low moisture content, $\geq$99%), NaOH, $\cdot KH_2PO_4$ (>99%), $K_2HPO_4 \cdot 12H_2O$ (>99%), N-hydroxy succinimide (NHS), ethanolamine and ethanol were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Loading sample buffer (LDS) was purchased from Invitrogen (Saint Aubin, France). Phosphate buffer solution (5 mM pH 7) was prepared and stored at 4 °C. All aqueous solutions were prepared using ultrapure water from Millipore system (Molsheim, France).

2.2. Bacterial cultures

All bacteria used in this work belong to the microorganism biosafety level 2 group and all safety considerations concerning this group were observed in the manipulation of these bacteria. All bacteria strains were cultured following the manufacturer's culturing guideline. Following overnight culture on Mueller Hinton agar plates, liquid culture was performed in Trypticase Soy Broth (TSB) at 37 °C with aeration by shaking, which allowed the growing mid-log-phase to be reached. The culture turbidity was adjusted to match a 0.5 McFarland standard and dilutions (10^8–10^2 CFU mL^{-1}) were performed in phosphate-buffered saline (PBS 1×), pH 7.4. Quantitative bacterial counts were performed in duplicate by placing 100 μL of the limited dilutions (10^3–10^2 CFU mL^{-1}) onto Mueller Hinton agar plates to confirm the bacterial concentrations. At the same time, the pure bacterial suspension was aliquoted in 100 μL and stored at −80 °C. Samples were rapidly thawed to 37 °C just before electrochemical measurements. Serial dilution in 5 mM pH 7 phosphate buffer of bacterial cultures (10^5–10^2 CFU mL^{-1}) was freshly prepared from a single-use aliquot to avoid freeze-thaw cell disruption.

2.3. Detection of Gram-negative bacteria by SDS-PAGE immunoblot analysis

Whole bacteria from the pure cultures and purified LPS were diluted in purchased LDS sample buffer and boiled for 10 min. The samples were loaded onto 12% NuPage Bis-Tris polyacrylamide gel from Invitrogen (Saint Aubin, France) and SDS-PAGE was carried out at 200 V for 45 min. Proteins and LPS were transferred to Immobilon, Millipore (Molsheim, France) PVDF membrane for 1 h15 min at 400 mA and the immunodetection was performed on the SNAP-id Protein Detection System from Millipore. After blocking with nonfat milk, the membrane was incubated with the primary antibody for 10 min. After washing, the secondary HRP-coupled antibody was added to the membrane and washed after 10 min of incubation. A final 10 min wash was performed and LPS

was visualized by using an enhanced chemiluminescent (ECL) reaction.

2.4. Preparation of the magnetic particle–antibody conjugates

The antibodies were covalently coupled to carboxylated superparamagnetic particles using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) chemistry. Magnetic particles (1 mg) were washed three times and resuspended in 100 μL activation buffer (Ademtech). EDC (6 mg/ml) was added and the mixture was incubated for 10 min under shaking at room temperature. After the addition of antibodies (40 μg), the beads were incubated for 2 h at room temperature. Ethanolamine (0.1%) was used to block the non-specific sites and incubated for 15 min at room temperature. The particles were attracted to the vial wall with a magnet and the supernatant was removed; then the particles were washed with storage buffer. The magnetic particle–antibody conjugates were resuspended in 100 μL phosphate buffer 20 mM pH 7 and stored at 4 °C until used.

2.5. Preparation of the immunosensor

Transducers were designed and produced at the Lashkaryov Institute of Semiconductor Physics (Kiev, Ukraine). The conductometric transducer consisted of two pairs of gold interdigitated thin-film electrodes (thickness: 150 nm), working and reference sensors, made by vacuum deposition on a ceramic substrate (Al_2O_3, 5 mm × 30 mm) (see scheme and photograph in Fig. S1).

Magnetic beads (200 nm) purchased from Ademtech (Pessac, France) were coated as described above. For the immunosensor preparation, 0.2 μL of nanoparticles functionalized with antibodies and with BSA, resuspended in 5 mM pH 7 phosphate buffer, were dropped on the working and the reference sensors. Magnets ($5 \times 5 \times 2$ mm^3, adherence strength: 650 g, Ademtech) were used to fix the functionalized beads onto the surface of the sensors. The modified sensors were stored in 20 mM phosphate buffer pH 7 at 4 °C until use.

2.6. Impedancemetric and microscopic characterization of captured bacteria

2.6.1. Electrochemical impedance spectroscopy measurements

In order to characterize the change of the interface impedance of the working electrode in the presence of increasing bacterial concentrations, electrochemical impedance measurements were recorded within the frequency interval 1 mHz–1 kHz (20 frequencies/decade) using an OrigaStat e100 potentiostat galvanostat impedancemeter from OrigaLys (Rillieux la Pape, France). The modified immunosensor was immersed in a glass cell containing 3 mL of 5 mM pH 7 phosphate buffer. The buffer was inoculated, under continuous stirring, with *E. coli* culture as a Gram-negative model, at final concentrations ranging from 1 to 10^5 CFU mL^{-1}. Measurements were carried out before and after capture at room temperature (20 ± 3 °C).

2.6.2. Scanning electronic microscopy (SEM)

The SEM images were obtained using a Quanta™ 250 microscope FEI (Eindhoven, The Netherlands). The dispersion of magnetic beads on the surface of the sensor was visualized using a sensor biofunctionalized according to the preparation procedure described in Section 2.5. After conductometric measurements, immunosensors giving efficient electrochemical responses in the presence of 1–10^4 CFU mL^{-1} were chosen for SEM analysis. The immunosensor was immersed in 3% glutaraldehyde solution for 45 min. Fixation is needed to preserve bacterial structure since bacteria contain proteins and a high proportion of water (Kaláb et al., 2008). Then, in order to prevent bacteria lysis, the immunosensor was dehydrated with ethanol by dipping it for 10 min in ethanol–water mixtures with increasing concentrations of ethanol: 20%, 40%, 60%, 80%, 100%.

2.7. Conductometry assays

The modified sensor was immersed in a glass cell containing 3 mL of 5 mM pH 7 phosphate buffer. The buffer was inoculated, under continuous stirring, with bacterial strains at final concentrations

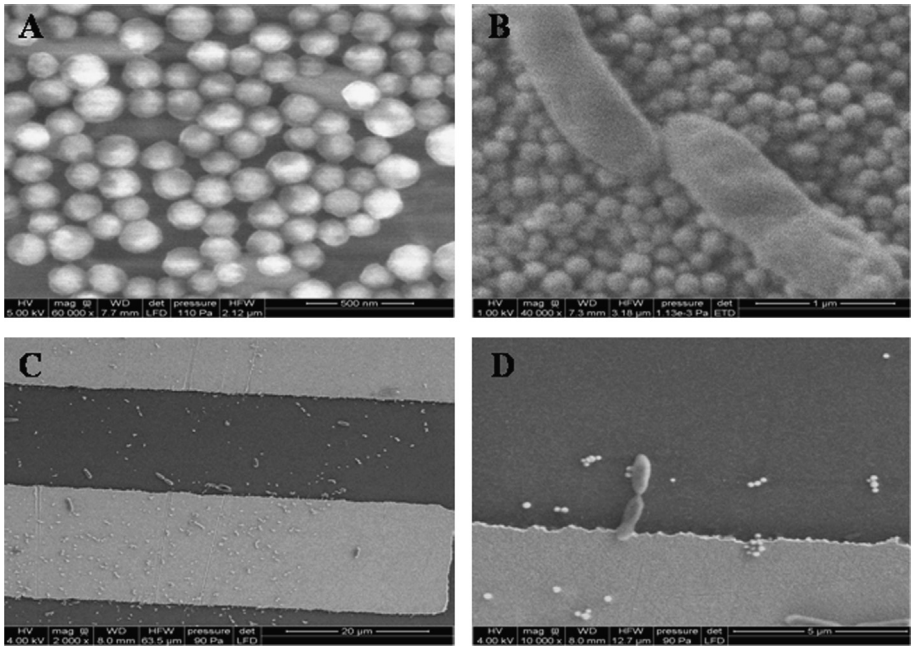

Fig. 1. SEM images of magnetic nanoparticles functionalized with the polyclonal anti-LPS antibodies (A) and after interaction with *E. coli* (B, C and D) on the surface of the interdigitated electrodes.

ranging from 1 to 10^5 CFU mL^{-1}. A low-frequency wave-form generator (SR830 Lock-in amplifier from Stanford Research Systems) was used. An alternating voltage of high frequency (10 mV amplitude, 100 kHz frequency) was applied, in order to be able to detect in real time, the change of ionic concentration in the boundary layer (Sheppard et al., 1996). The conductometric measurements during bacterial capture were performed in a differential mode. The inoculation was performed after reaching the baseline behavior of the sensor which is required for accurate measurements (Jacobs et al., 1994). Measurements were performed at room temperature (20 ± 3 °C).

3. Results and discussion

3.1. EIS and SEM characterization of bacterial capture on the biosensor surface

The biosensor was designed by immobilizing anti-LPS antibodies on the surface of activated magnetic nanoparticles. Non-faradic impedance measurements were performed to monitor the change of electrical properties at the surface of the interdigitated microelectrodes before and after bacteria capture (Radke and Alocilja, 2005). In fact, the capture of bacteria with a highly insulating cell membrane (Ehret et al., 1997; Yang and Bashir, 2008) results in a change of the interface impedance. Nyquist plots, presented in Fig. S2, show a variation of the impedance before and after *E. coli* binding at a concentration of 10^3 CFU mL^{-1}. It has been demonstrated that the biomaterial mass change due to the attachment of bacteria on the electrode surface generates a variation of the impedance values at low frequencies (Ben-Yoav et al., 2011). This variation is due to the accumulation of non-conducting and negatively charged bacteria on the working electrode surface by specific immunocapture. In the present work, when the *E. coli* concentration in contact with the immunosensor increases, the quantity of trapped bacteria on the immunosensor surface increases, leading to a linear variation of the impedance module (frequency: 223 mHz) with a sensitivity of 0.2 MΩ C-FU^{-1} mL (data not shown).

SEM images of these particles on the surface of the interdigitated electrodes are shown in Fig. 1 before and after reaction with *E. coli*. The magnetic beads have a spherical shape with an average diameter of 200 nm as shown in Fig. 1A. They are uniformly distributed over the sensor and form a matrix on which bacteria were captured during interaction with anti-LPS antibody (Fig. 1B). In Fig. 1C and D, SEM images of a part of the sensor surface with a low coverage are presented to show the interaction of bacteria with the functionalized magnetic particles. These images confirm the capture of whole bacteria.

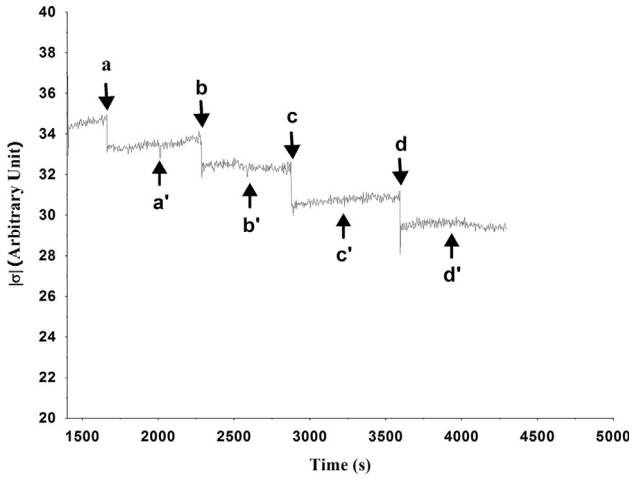

Fig. 2. Electrochemical response of the conductometric immunosensor based on polyclonal anti-LPS antibody functionalized magnetic nanoparticles in the presence of *E. coli* concentrations (a): 1, (b): 2, (c): 4, (d): 6, $\times 10^3$ CFU mL^{-1} or *S. epidermidis* concentrations: (a′): 1, (b′): 2, (c′): 4, (d′): 6×10^3 CFU mL^{-1}.

3.2. Analytical parameters of the immunosensor

3.2.1. Detection of E. coli

In the first part of this work, *E. coli* was used as a Gram-negative bacterium to evaluate the performance of the conductometric immunosensor. Monoclonal and polyclonal anti-LPS antibodies were chosen for the functionalization of magnetic nanoparticles. The sensitivity and the response-time of the immunosensor depend on the specific antigen–antibody reactions in the presence of Gram-negative bacteria. In order to design a multi-target bacterial immunosensor, polyclonal antibodies were preferred.

To demonstrate the selectivity of our immunosensor, a specificity control was performed by recording the electrochemical response of the biosensor to increasing concentrations of Gram-positive *S. epidermidis* (1×10^3 to 6×10^3 CFU mL^{-1}). *E. coli*, as a positive control, was spiked into the electrochemical cell at the same concentrations (Fig. 2). The change in conductance in the presence of Gram-positive bacteria was not significant while in the case of Gram-negative bacteria, a real time decrease of the conductance was observed, that could be assigned to the variation of the ionic conductance during capture of Gram-negative bacteria. The biosensor is highly specific to selected Gram-negative antigens even in samples with multiple bacteria strains. The use of BSA functionalized magnetic particles for the construction of a control sensor demonstrated that the conductometric variation was due to the bacteria–antibody interaction, excluding any adsorption phenomenon on the non-specific electrode surface (Fig. S3).

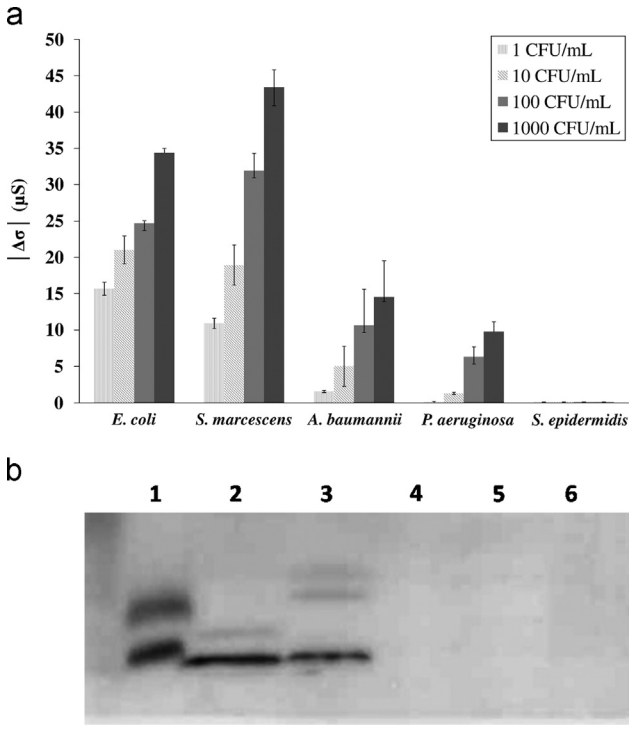

Fig. 3. (a) Conductometric response of the immunosensor based on polyclonal anti-LPS antibody functionalized magnetic nanoparticles in the presence of Gram-negative bacteria. (a) Immunoblot analysis of Gram-negative bacteria, 1: purified LPS, 2: *E. coli*, 3: *S. marcescens*, 4: *A. baumannii*, 5: *P. aeruginosa*, 6: *S. epidermidis* (Gram-positive specificity control).

Vashist et al. (2006) demonstrated that a conduction decrease may occur due to the inactivation of certain charged conducting groups of the IgG molecules after the recognition reaction to the corresponding anti-IgG. In the presence of bacteria which present a negative surface net charge coming from the negative lipids and lipopolysaccharide (LPS) molecules in the outer leaflet of the outer membrane (Domingues et al., 2008), the resulting electrode surface net charge will change, also changing the local concentration of counter-ions.

To demonstrate that the immunosensor was able to detect whole bacteria cells, the electrochemical response was monitored in the presence of *E. coli* lysates obtained by heat-treatment for 5 min at 100 °C. Where *E. coli* lysates were present, no electrochemical response was observed (data not shown).

In order to determine the working range of the biosensor, the conductance response was monitored, varying the concentration of *E. coli* in the reaction solution from 1 to 10^5 CFU mL^{-1}. Two linear ranges, 1–10^4 and 10^4–10^5 CFU mL^{-1}, were noticed. A saturation phase occurred at concentrations higher than 10^5 CFU mL^{-1} (data not shown). The immunosensor showed a fully reproducible linear range at 1–10^3 CFU mL^{-1} with an estimated sensitivity of 7 μS per decade of CFU mL^{-1} (presented as a histogram in Fig. 3A). This is of practical importance knowing that the sensitivity of methods that could be used at the point of transfusion would most likely be in the range of 10^2–10^3 CFU mL^{-1} (Brecher et al., 2000).

The change in conductivity was nearly instantaneous with response stability after 2 min, on average. The measurements were performed using a minimum sample volume of 3 mL which is necessary to ensure the representation of very low levels of bacteria.

3.2.2. Specificity control of the immunosensor

In the second part of this work, the ability of the polyclonal anti-LPS antibody-based immunosensor to detect the presence of bacteria was tested with Gram-negative strains among clinically relevant bacteria (*E. coli*, *S. marcescens*, *A. baumannii* and *P. aeruginosa*) and a Gram-positive strain (*S. epidermidis*) as a specificity control that were intentionally spiked into the measuring cell at increasing final concentrations from 1 to 10^3 CFU mL^{-1}. The histograms shown in Fig. 3A represent the variation of the absolute value of conductivity, $I\Delta\sigma I$, for different concentrations of each bacterial strain (1, 10, 10^2, 10^3 CFU mL^{-1}). The average values of $\Delta\sigma$ and the standard deviations were calculated using data recorded on assays performed in triplicate for *E. coli* and in duplicate for other strains and separately for each bacterium. The relative standard deviation was 3.64% for *E. coli*, 6.86% for *S. marcescens*, 33.79% for *A. baumannii* and 13. 94% for *P. aeruginosa*. The change in conductance was proportional to the logarithm of bacteria concentration in the range of 10–10^3 CFU mL^{-1} (*E. coli*: 7 μS per decade, *S. marcescens*: 11 μS per decade, *A. baumannii*: 5 μS per decade, *P. aeruginosa*: 3.3 μS per decade). The measured detection limit was down to 1 CFU mL^{-1} for *E. coli* and *S. marcescens* and 10 CFU mL^{-1} for *A. baumannii* and *P. aeruginosa* respectively. The difference in sensitivity could be assigned to the affinity between the polyclonal antibody and the LPS of each bacterial strain. LPS is composed of three distinct regions: the lipid A, the core oligosaccharide and the O-polysaccharide chain. This O-chain is extremely variable and constitutes the majority of antigenic determinant (Poxton, 1995, Erridge et al, 2002, Caroff and Karibian, 2003). No antibody is able to detect with a same sensitivity all different LPS biomolecules of Gram-negative bacteria. In order to improve the sensitivity of the immunosensor, a cocktail of anti-LPS antibodies directed against various bacteria families will be used as bioreceptors in a future project. The electrochemical detection was compared to the immunoblot analysis of these bacterial cultures using the same polyclonal anti-LPS antibody (Fig. 3B). The conductometric immunosensor allowed the direct detection of low concentrations of *P. aeruginosa* and *A. baumannii* strains that were undetectable using the standard immunoblot methods. This point could be explained by the preparation procedure used for immunoblot test that could stress these bacteria, which is not the case for the real time conductometric measurements.

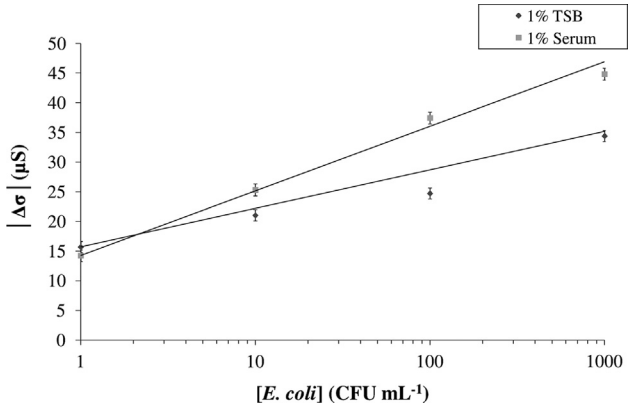

Fig. 4. Calibration curves of the microconductometric immunosensor based on polyclonal anti-LPS antibody functionalized magnetic nanoparticles in the presence of 1% TSB and 1% serum in 5 mM phosphate buffer pH 7. The reported values are the mean of duplicated measurements for each bacterium concentration.

In the case of a mixture of different Gram-negative strains, the total concentration could be surestimated by choosing the sensitivity for *P. aeruginosa*. This immunosensor should be a warning system of detection with a detection limit of 10 CFU mL^{-1} of Gram-negative bacteria that should be confirmed using classical techniques.

In order to demonstrate the potential use of the immunosensor for blood monitoring, further experiments were performed in diluted serum.

3.2.3. Detection of bacteria in serum

The performance of the immunosensor was evaluated with regard to potential interference of other components in the test medium. For this purpose, the electrochemical response of the biosensor was monitored in the presence of increasing concentrations of *E. coli* under the same conditions but in three test media: 5 mM pH 7 phosphate buffer, 1% TSB and 1% serum. Serum was chosen rather than blood to limit the effect of the immunological and enzymatic components. In addition, it has been reported that the presence of albumin in blood acts as a blocking agent for the antibody–antigen interactions and so reduces sensor sensitivity. In our studies, TSB and serum dilutions were performed in 5 mM phosphate buffer, pH 7. Due to the complexity of these samples, they were diluted without further pre-treatment in order to minimize background noise and to avoid interdigitated electrode saturation. The same medium was used to prepare dilutions from bacterial cultures and to equilibrate the biosensor for the initial baseline determination. The variation $I\Delta\sigma I$ was plotted against the logarithm of the bacterial concentration. In the presence of *E. coli*, a conductance variation proportional to the log of *E. coli* concentrations is observed (Fig. 4). Results showed that the conductometric response of the biosensor was not affected by the presence of 1% TSB (7 μS per decade). No significant effect of the interferences usually found in blood serum was noticed on the conductometric response of the immunosensor (10 μS per decade). The enhanced sensitivity in 1% serum could be explained by the presence of various ions in the serum that should enhance the bacterial affinity.

3.2.4. Comparison of the performance of our sensor with that of relevant previous electrochemical sensors

The relevant previous electrochemical biosensors for the detection of bacteria, mainly focused on detection of *E. coli*, are presented

in Table 1. The immunosensor designed in this work presents the lowest detection limit and the response time is in the lowest range. Moreover, our immunosensor is based on an anti-LPS antibody, which is quite original, allowing selective detection of Gram-negative bacteria, including clinically relevant bacteria, which has never been performed before, using electrochemical transducers. Moreover, our immunosensor has been shown to be able to detect strains that were not detected using standard immunoblot methods.

4. Conclusion

The purpose of this study was the construction of a conductometric biosensor for the highly sensitive and rapid detection of bacterial contamination in a biological sample. Our first results have shown that the designed biosensor allowed direct, real-time, detection of *E. coli* cultures from 1 to 10^3 CFU mL^{-1}. The performance of the immunosensor was evaluated in the presence of reference and clinical bacteria. There was a close linear correlation between the conductometric signal and the log of the bacterial spiking concentration. The ability of the magnetic nanoparticle-based conductometric immunosensor to detect *P. aeruginosa* and *A. baumannii*, not detectable through immunoblot, was demonstrated.

The performance of this designed immunosensor will be tested using different antibodies or antimicrobial peptides for the generic detection of Gram-negative and Gram positive bacteria. Further practical use of this biosensor is being investigated through the design of an integrated laboratory-type platform on a chip for detection in real biological samples.

Acknowledgments

This work was financially supported by the Etablissement Français du Sang APR 2010-2012, RapidBact Project. The authors thank J.-M Girard (OrigaLys company, Rillieux la Pape, France) for set-up supplying and fruitful discussion.

Appendix A. Supplementarey matrial

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.11.016.

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