

Microconductometric Detection of Bacterial Contamination

^{1,2} Sarra EL ICHI, ¹ Fanny LEON, ¹ Ludivine VOSSIER,
³ Hélène MARCHANDIN, ² Abdelhamid ERRACHID,
^{2*} Nicole JAFFREZIC-RENAULT, ¹ Joliette COSTE,
¹ Chantal FOURNIER-WIRTH, ⁴ Jan KREJČÍ, ⁴ Radka KUČEROVÁ,
⁴ Tomáš KREJČÍ

¹ Etablissement Français du Sang, Laboratoire TransDiag, Parc Euromédecine, 392 Avenue
Professeur Jean-Louis Viala, 34184 Montpellier, France

² Université de Lyon, Institut des Sciences Analytiques, UMR CNRS 5280,
5 Rue de La Doua, 69100 Villeurbanne, France

³ CHRU Montpellier, Laboratoire de Bactériologie UMR 5119, Université Montpellier 2,
Place Eugène Bataillon, 34095 Montpellier, France

⁴ BVT Technologies a.s, Hudcova 78c, 61200 Brno, Czech Republic

¹ Tel.: +33437423558

E-mail: nicole.jaffrezic@univ-lyon1.fr

Received: 31 December 2012 / Accepted: 10 August 2013 / Published: 26 May 2014

Abstract: Several approaches can be used for the electrochemical detection of bacterial contamination. Their performance can be assessed by the ability to detect bacteria at very low concentrations within a short-time response. We have already demonstrated that a conductometric biosensor based on interdigitated thin-film electrodes is adapted to detect bacteria in clinical samples like serum and compatible with microfluidic fabrication. The type of interdigitated microelectrodes influences the performance of the biosensor. This was shown by the results obtained in this work. A magnetic-nanoparticles based immunosensor was designed using gold screen-printed electrodes. The immunosensor was able to specifically detect *E. coli* in the range of 1-10³ CFU mL⁻¹. The new transducer offered a larger active sensing surface with a lower cost and a robust material. Accuracy of the conductance value was enhanced by differential measurements. The immunosensor is compatible with a microfluidic system. Copyright © 2014 IFSA Publishing, S. L.

Keywords: Bacteria, Interdigitated electrodes, Immunosensor, Conductometry.

1. Introduction

Unvoluntary or voluntary (bioterrorism) development of pathogenic bacteria threat is a global concern. Different types of matrices may be contaminated: environmental, biomedical, food, etc.

The complexity of these matrices and experimental difficulties results in limitation of sensitivity and time of analysis of common analytical methods such as microscopy, flow cytometry, culturing techniques, etc. Enzyme- and immunosensors emerged as promising alternatives for detection of pathogenic

bacteria in a variety of fields. Miniaturized platforms are highly suitable especially in the medical field [1, 2].

Over the past few years, electrochemical detection accompanied by progress in the nanoscale and microfluidics fabrication has been reported as an interesting alternative to conventional detection methods of microorganisms in food and environmental samples [3-5]. A great interest has arisen for the electrochemical detection in the clinic [6] and environmental analysis [7]. The importance of biosensors and related recent development has been reviewed by Ivnitski and collaborators [8]. Interdigitated design has attracted great attention since it brings enhancement of the sensor's performance compared to conventional electrodes. The advantages of interdigitated structures have been reported in previous works [9]. Reproducible shapes and sizes of electrodes can be obtained using photolithography. Electrochemical characteristics and possible applications of interdigitated electrodes have been described in literature [10-12]. The material used for fabrication and the dimensions of patterned microelectrodes and their geometries influence the diffusion process and the sensitivity [13]. The robustness and the active sensor area are among the differentiating criteria of the interdigitated microelectrodes [14]. It is interesting to use low price and disposable electrodes. In this case, a great reproducibility is needed to avoid fluctuations in electrochemical measurements. This demand was solved by differential conductivity measurement.

Conductometric measurements being not selective, the selectivity is ensured by the ability of an antibody to recognize only the target antigen in the presence of other interfering components or microorganisms in the sample solution. There are several coupling strategies to conjugate antibodies to the electrode surface. Among interesting supports for biomolecules, nanoparticles offer many advantages such as a suitable size, different functionalization methods and many possible applications (drug delivery, biosensing, bioimaging, etc.) [15]. Antibody-coated paramagnetic beads were used for amperometric detection of *E. coli*. The method allowed a detection limit of 20 CFU mL⁻¹ within 7 h [7].

In a previous work, we demonstrated the performance of a new conductometric biosensor based on antibody-coupled nanoparticles attached to gold interdigitated electrodes via a magnetic field [16, 17]. This system allowed detection of bacteria at low concentrations (1 to 10³ CFU mL⁻¹). In this work, screen-printed electrodes replaced the previous ones in the design of the conductometric immunosensor. Their performance is evaluated with regards to sensitivity and detection limit. The new system integrates two interdigitated structures. The first one measures the background phenomena. The second one measures the presence of microorganisms and differential measurements are performed.

2. Experimental Part

2.1. Microorganism, Biomaterials and Chemicals

All bacteria strains, *Escherichia coli* (CIP 76.24), *Serratia marcescens* (CIP 103235), 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, ≥ 99 %), NaOH, KH₂PO₄ (> 99 %), K₂HPO₄·12H₂O (> 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. Bacteria Culturing

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 (10⁸-10² CFU mL⁻¹) were performed in phosphate-buffered saline (PBS 1X), pH 7.4. Quantitative bacterial counts were performed in duplicate by placing 100 µL of the limited dilutions (10³-10² 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 conductometric measurements.

2.3. The Screen Sensor Preparation

The differential conductometric sensor structure was prepared by screen printing [18]. The conductor from silver and the active gold layer were printed on the alumina ceramics. The structure was fired at 850 °C. Then the final dielectric protective layer was printed. The sensors contain the common electrode which is connected to the middle contact and two independent electrodes connected to the left and right contact. The dielectric layer defines the size of the active surface and the cell constant. The batch of printed sensors was measured and the parameters and their standard deviation were analyzed. The structure of the sensor and designed dimensions are in Fig. 1.

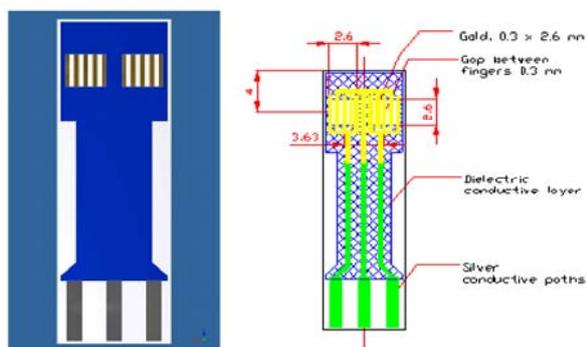


Fig. 1. The structure of the differential microconductometric sensor.

The measured real dimensions are reported in Table 1. The basic dimensions of the sensors can be prepared with sufficient reproducibility. This is the basic condition which enables to produce robust sensors.

Table 1. Actual dimensions of the sensor and standard deviations.

Dimension	Value	Standard deviation (SD)	SD [%]
Left window size	2.63 × 2.62 mm	0.04 mm	1.5 %
Right window size	2.67 × 2.62 mm	0.025 mm	1.0 %
Window area	6.95 mm ²	0.12 mm ²	2 %
Electrode width	0.300 mm	0.015 mm	5 %
Electrode length	2.62 mm	0.04 mm	1.5 %
Left active electrodes surface	2.33 mm ²	0.12 mm ²	5.3 %
Left active electrodes surface	1.66 mm ²	0.14 mm ²	9.0 %
Right active electrodes surface	2.44 mm ²	0.09 mm ²	3.5 %
Right common electrodes surface	1.63 mm ²	0.08 mm ²	5.0 %

2.4. Preparation of the Biosensor

Magnetic beads (200 nm, Ademtech France) were coupled via EDC/NHS activation method with the selected anti-LPS antibodies. The magnetic beads

(1 %) were rinsed with cold 25 mM MES-NaOH buffer pH 5. EDC/sulfo-NHS (30 mM/30 mM in 25 mM MES-NaOH buffer pH 5) was used for the activation reaction during 2 h. Then, they were rinsed and resuspended into 5 mM phosphate buffer pH 7. Two volumes of activated magnetic particles (5 μ L) of activated beads) and with BSA (1 mg mL⁻¹) during 2 h under stirring. The magnetic beads were reacted with 0.1 % ethanolamine during 20 min for the blocking of unspecific binding sites. 0.4 μ L of functionalized nanoparticles with antibodies and BSA, resuspended in 5 mM phosphate buffer pH 7.4, were dropped respectively on the working and the reference sensors. Magnets (5×5×2mm, adherence strength: 650 g, Ademtech) were used to fix the functionalized beads on the surface of the electrodes by a magnetic field. The modified sensors were stored in 5 mM phosphate buffer pH 7 at 4 °C until use.

2.5. Differential Conductometry Measurements

The modified sensor was emerged in a glass vessel containing 3 mL of 5 mM phosphate buffer pH 7.4. The buffer was inoculated, under continuous stirring, with bacterial strains at final concentrations ranging from 1 to 10³ CFU mL⁻¹. A low-frequency wave-form generator (SR830 Lock-in amplifier from Stanford Research Systems) was used. An alternating voltage (10 mV amplitude, 100 kHz frequency) as applied. The conductometric measurements were performed in a differential mode.

The response was recorded after reaching baseline behaviour of the sensor which is required for accurate measurements [19].

Measurements were performed at room temperature (20 ± 3 °C). Bacterial cultures stored at -80 °C were thawed quickly by immersion in water at 37 °C. Serial dilution in 5 mM phosphate buffer pH 7.4 of bacterial cultures (10⁵ to 10² CFU mL⁻¹) was freshly prepared from a single-use aliquot to avoid freeze-thaw cell disruption.

3. Results and Discussion

3.1. Characterization of the Micro-Conductometric Sensor

Frequency scans were performed over the range 100 Hz–100 kHz with signal amplitude of 10 mV. The conductometric response (real part of impedance) of the bare screen-printed electrodes was monitored in presence of increasing KCl concentrations, as presented in Fig. 2.

The optimal operating frequency was higher than 10 kHz, 100 kHz was chosen for further experiments.

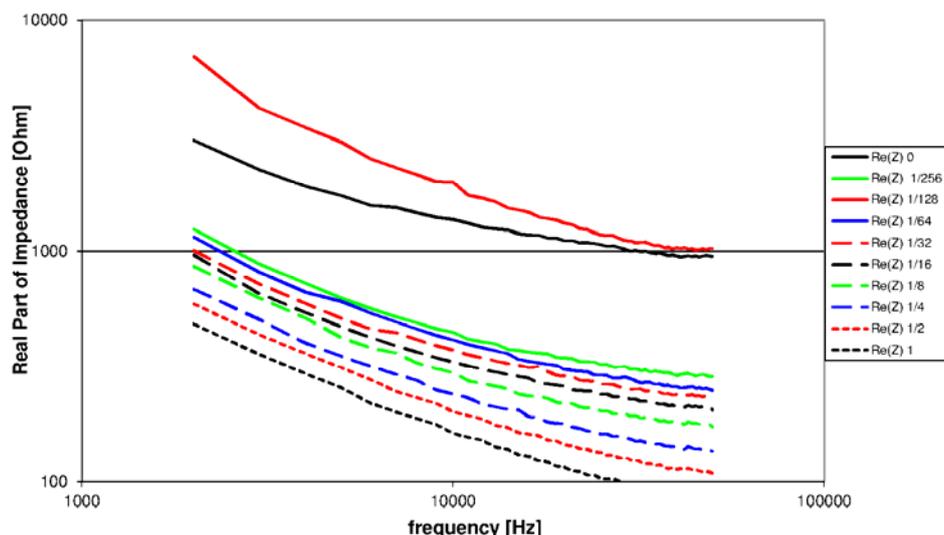


Fig. 2. Variation of real part of impedance of the microconductometric sensor versus frequency for different molar concentrations of KCl.

3.2. Analytical Performances of the Microconductometric Immunosensor

E. coli was used as gram-negative bacteria to evaluate the performance of the conductometric immunosensor. The biosensor was equilibrated in 25 mM phosphate buffer pH 7 until reaching signal stability then considered as a baseline. In order to demonstrate the selectivity of the biosensor, *S. epidermidis*, as a control strain, was injected at a final concentration of 10^3 CFU mL⁻¹ prior to spiking *E. coli* into the electrochemical cell. The immunosensor responded only to *E. coli* injections.

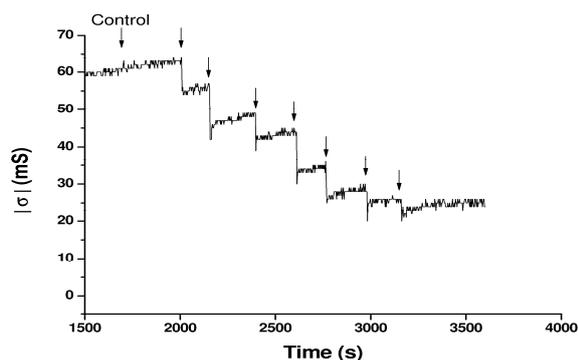


Fig. 3. Electrochemical response of the microconductometric immunosensor in presence of *E. coli* concentrations from 1 to 10^3 CFU mL⁻¹, control: 10^3 CFU mL⁻¹ *S. epidermidis*.

Fig. 3 shows a typical electrochemical response of the conductometric immunosensor in presence of increasing concentrations of *E. coli*. In presence of bacteria, the electrochemical response was rapid reaching signal stability within 2 min in average. The

increasing *E. coli* concentrations resulted in decreasing conductance. Values of these variations were determined by subtracting the baseline reference. Calibration curve was obtained by plotting the variation of the absolute value of conductance $|\Delta\sigma|$ against the logarithm of the bacterial concentration, as presented in Fig. 4.

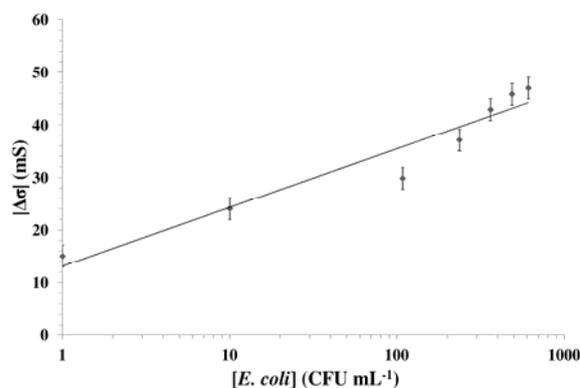


Fig. 4. Calibration curve of the microconductometric immunosensor: Variation of the absolute value of conductance $|\Delta\sigma|$ against the logarithm of the bacterial concentration.

The dynamic range of 1 - 10^3 CFU mL⁻¹ is linear with an estimated sensitivity of 11.16 mS CFU⁻¹ mL. The measured detection limit was as low as 1 CFU mL⁻¹. This value is similar to the one reported in a previous work [17].

4. Conclusion

Our first results have shown that screen-printed interdigitated electrodes are reliable tools for a

sensitive and rapid conductometric detection of bacterial contamination. The active surface of the sensor was increased by using antibody-coated magnetic nanoparticles. The use of magnets simplified the attachment of nanoparticles on the electrode surface and prevented antibody denaturation. The immunosensor allowed real-time and specific detection of *E. coli* cultures from 1 to 10^3 CFU mL⁻¹. Among the advantages of using this low-cost single-use biosensor, the active surface area is bigger than similar devices, no need for cleaning steps and enhanced accuracy by using one reference electrode. Furthermore, the designed biosensor is compatible with microfluidic systems which have among their advantages the reduction of the sample volume. The immunosensor will be evaluated with different bacteria and its efficiency in real samples will be investigated.

Acknowledgements

The authors thank 7th FP of European Union (INFULOC project No. 230749) for their financial support. BVT Company thanks Czech Ministry of Industry for their support through DLC project No. FR-TI1/118.

References

- [1]. C. Fournier-Wirth, J. Coste, Nanotechnologies for pathogen detection: Future alternatives, *Biologicals*, Vol. 38, 2010, pp. 9-13.
- [2]. C. Fournier-Wirth, N. Jaffrezic-Renault, J. Coste, Detection of blood-transmissible agents: can screening be miniaturized, *Transfusion*, Vol. 50, Issue 9, 2010, pp. 2032-2045.
- [3]. P. Leonard, S. Hearty, J. Brennan, L. Dunne, J. Quinn, T. Chakraborty, R. O'Kennedy, Advances in biosensors for detection of pathogens in food and water, *Enzyme and Microbial Technology*, Vol. 32, 2003, pp. 3-13.
- [4]. J. Heo, S. Z. Hua, An overview of recent strategies in pathogen sensing, *Sensors*, Vol. 9, 2009, pp. 4483-4502.
- [5]. L. Zhang, J. Xu, L. Mi, H. Gong, S. Jiang, Q. Yu, Multifunctional magnetic-plasmonic nanoparticles for fast concentration and sensitive detection of bacteria using SERS, *Biosensors and Bioelectronics*, Vol. 31, Issue 1, 2012, pp. 130-136.
- [6]. Y. Wang, H. Xu, J. Zhang, G. Li, Electrochemical sensors for clinic analysis, *Sensors*, Vol. 8, Issue 4, 2008, pp. 2043-2081.
- [7]. I. H. Boyaci, Z. P. Aguilar, M. Hossain, H. B. Halsall, C. J. Seliskar, W. R. Heineman, Amperometric determination of live *Escherichia coli* using antibody-coated paramagnetic beads, *Analytical and Bioanalytical Chemistry*, Vol. 382, 2005, pp. 1234-1241.
- [8]. D. Ivniński, I. Abdel-Hamid, P. Atanasov, E. Wilkins, Biosensors for detection of pathogenic bacteria, *Biosensors and Bioelectronics*, Vol. 14, 1999, pp. 599-624.
- [9]. N. Jaffrezic-Renault, S. V. Dzyadevych, Conductometric microbiosensors for environmental monitoring, *Sensors*, Vol. 8, Issue 4, 2008, pp. 2569-2588.
- [10]. Y. Iwasaki, M. Morita, Electrochemical measurements with inter-digitated array microelectrodes, *Current Separations*, Vol. 14, Issue 1, 1995, pp. 2-8.
- [11]. A. E. Cohen, R. R. Kunz, Large-area interdigitated array microelectrodes for electrochemical sensing, *Sensors and Actuators B*, Vol. 62, 2000, pp. 23-29.
- [12]. J. Hubalek, J. Krejci, Correction factors of IDEs for precise conductivity measurements, *Sensors and Actuators B*, Vol. 91, Issue 1-3, 2003, pp. 46-51.
- [13]. A. M. Sampson, E. T. K. Peterson, I. Papautsky, in *Proceedings of SPIE of the Conference on Microfluidics, BioMEMS, and Medical Microsystems VI*, San Jose, California, USA, 21-22 January 2008, Vol. 6886, pp. 6886-35.
- [14]. J. Krejci, R. Sejnohova, V. Hanák, H. Vranova, New Perspectives in Biosensors Technology and Applications, Pier Andrea Serra (Ed.), *InTech*, 2011.
- [15]. M. De, P. S. Ghosh, V. M. Rotello, Applications of nanoparticles in biology, *Advanced Materials*, Vol. 20, 2008, pp. 4225-4241.
- [16]. M. Hnaiein, W. M. Hassen, A. Abdelghani, C. Fournier-Wirth, J. Coste, F. Bessueille, D. Leonard, N. Jaffrezic-Renault, A conductometric immunosensor based on functionalized magnetite nanoparticles for *E. coli* detection, *Electrochemistry Communications*, Vol. 10, 2008, pp. 1152-1154.
- [17]. S. El Ichi, F. Leon, L. Vossier, H. Marchandin, A. Errachid, J. Coste, N. Jaffrezic-Renault, C. Fournier-Wirth, Microconductometric immunosensor for label-free and sensitive detection of Gram-negative bacteria, *Biosensors and Bioelectronics*, 54, 2014, pp. 378-84.
- [18]. M. R. Haskard, K. Pitt, Thick-Film technology and application, *Electrochemical Publications Ltd*, 1997.
- [19]. P. Jacobs, J. Suls, W. Sansen, Performance of a planar differential-conductivity sensor for urea, *Sensors and Actuators B*, Vol. 20, 1994, pp. 193-198.