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Detection of pathogenic *Staphylococcus aureus* bacteria by Electrochemical Impedance

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Abstract

The timely detection of pathogens is a subject of great importance. In this work, the objective is to elaborate an immunosensing system for detection and quantification of *Staphylococcus aureus*. Poyclonal *anti-S. aureus* are immobilized onto gold electrodes via chemical bond formation between antibody amino groups and a carboxylic acid containing self-assembled molecular monolayer. The evaluation of the developed immunosensor performance was accomplished through the monitoring of the electron-transfer resistance detected by electrochemical impedance spectroscopy in the presence of $[Fe(CN)_6^{3-}]/[Fe(CN)_6^{4-}]$ as redox probe. A low detection limit of 10 fcu/ml and a linear range up to 10^7 cfu/ml were obtained.

Key words: *Staphylococcus aureus*, self-assembled molecular monolayer, immunosensor, electrochemical impedance spectroscopy.

Introduction

The detection of pathogenic bacteria remains a challenging and important issue for ensuring food safety and security, for controlling water and soil pollution, and for preventing bioterrorism and nosocomial diseases [1]. In France the bacterial pathogen *Staphylococcus aureus* is the second major cause of food poisoning outbreaks after *Salmonella* [2].
Biosensors techniques are particularly attractive for the detection and identification of pathogenic microorganisms due to their potential sensitivity and specificity [3]. Several detection techniques have been employed for identification and /or monitoring of bacterial contamination in food. Electrochemical impedance spectroscopy (EIS) continues to be a topic of recent interest [1,4,5].
On the other hand, the immobilization of biomolecules on transducers is a potentially important prerequisite for the fabrication of immunosensor. Various recent works have focused on self-assembled monolayers (SAMs) thanks to its simplicity, versatility, reproducibility and high level of molecular ordering in the electrode modification [6]. In fact Alkanethiols terminated with functional groups, such as a carboxylic acid and amine, are strongly chemisorbed with high order on various metal surfaces such as gold.

Experimental

1- Reagents

Polyclonal antibodies (developed in rabbit) against *Staphylococcus aureus* were obtained from BIOtech[RDP] (Sfaxe, Tunisia).
16-Mercaptohexadecanoic acid, N-(3 Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), potassium Ferrocyanide ($K_4Fe(CN)_6$), potassium ferricyanid ($K_3Fe(CN)_6$), Phosphate Buffered Saline (PBS), ethanolamine, 30% hydrogen peroxide (all from Sigma), hydrochloric acid (Prolabo), and Ethanol was obtained from Fluka (purity>99%). All solutions were prepared with deionized Milli-Q water.

2- Bacterial Culture

S. aureus (ATCC25923) cells were grown in tryptic soy broth [TSB, (Difco)] or on TSB agar plates. High titer of bacteria suspension was prepared as follows: liquid culture mediums were inoculated by 100 μL of preculture solution and cultivated at 37°C for 18–24 h. Centrifuge the cells at 6400 rpm for 5min, wash the cells

twice, and resuspend the cells in sterile phosphate-buffered saline (PBS). Determine the viable cells and bacterial concentration with spread-plate technique. The optical density OD of the bacterial culture has been measured for the determination of bacterial growth stationary phase.

3- Instrumentation

Electrochemical measurements were performed with a three-electrode cell, comprising of a saturated calomel electrode (SCE) as the reference electrode, a platinum plate as auxiliary electrodes and functionalized gold substrate act as working electrodes.

Cyclic voltammetry (CV) and impedance measurements were carried out with a Potentiostat/galvanostat Radiometer Analytical (Voltalab40-PGZ301).

4- Procedure

The Au electrodes were ultrasonically cleaned by acetone and 5 min in a mixture piranha (H_2O_2/HCl:3/7), between and after these treatments, the gold electrodes were rinsed thoroughly with ultrapure water, then rinsed with absolute ethanol and dried in a flow of nitrogen. After cleaning, gold substrates were immersed into a thiol solution (10mM) dissolved in ethanol for 12 h. The SAMs layer of thiols was activated by incubation in a mixture of 0.1M EDC and 0.1M NHS for 1 h. After that, the incubation of Polyclonal antibodies against *S. aureus* (ATCC 25923) suspension in PBS at 0.2 mg/ml for 1 h, the inbound NHS esters were blocked by flowing ethanolamine 10mM for 20 min (figure1).

In order to detect *S. aureus* by direct essay, the prepared surface sensor was incubated in different concentration of *S. aureus* (10 to 10^9 CFU/ml). After each incubation, impedance measurement was effected to check sensitivity of immunosensor elaborated.

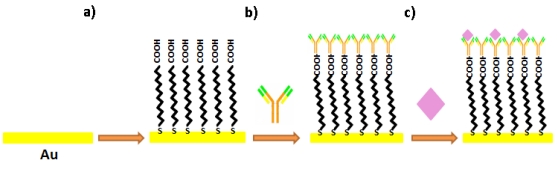

Fig. 1. Structure and components of the immunosensor: a) self-assembled monolayers b) antibody binding and c) the detection o bacteria.

Results and discussion

A carboxylic acid derivative was self-assembled on the electrode surface and activated by reaction with a carbodiimide. Subsequently, the in antibody was attached covalently to the surface via amide bonding [6].

The quality of self-assembled monolayers formed onto electrode surfaces and the antibody binding were investigated by cyclic voltammetry and impedance spectroscopy in the presence of the redox probe Ferrocyanide. Figure 2 show how the electric current through the electrode is effectively reduced in the presence of the monolayers

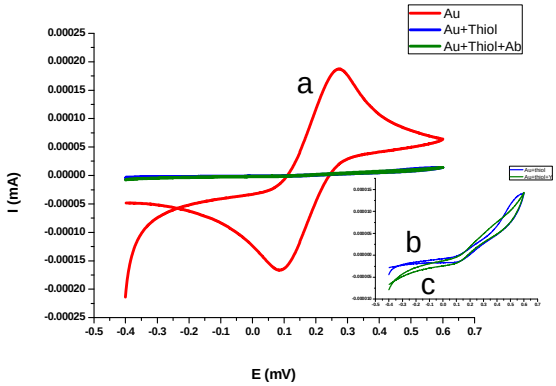

Fig. 2. Cyclic voltammograms recorded in the presence of $[Fe(CN)_6^{3-}]/[Fe(CN)_6^{4-}]$ as redox probe at scan rates of 100mV/s, a) bare Au b) gold electrode modified with a SAMs and c) b + covalently bonded antibody.

Nyquist plots of impedance spectra obtained for the different fictionalization steps show a remarkable variation of the charge transfer resistance R_{CT} (figure3), in fact, the electron transfer resistance of redox probe increased in large value after SAM modification and immobilization of Anti-*S. aureus* antibody.

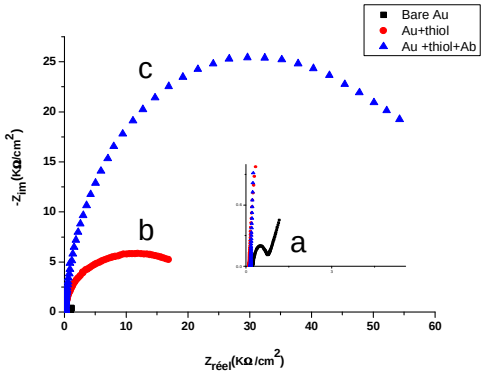

Fig. 3. Nyquist plots of impedance spectra's recorded in the presence of $[Fe(CN)_6^{3-}]/[Fe(CN)_6^{4-}]$ as redox probe at a frequency range between 0.1Hz and 100KHz, a) bare Au b) gold electrode modified with a SAMs and c) b + covalently bonded antibody.

Nyquist diagram for the faradic impedance measurement of modified gold electrode in the presence of varying concentration of target bacteria is given in figure 4 (A), the most

distinctive differences in magnitude of impedance were observed at the low frequency.

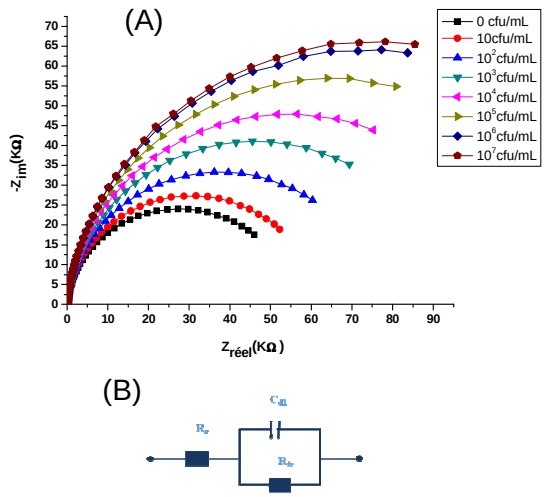

Fig. 4. (A) Nyquist plots of impedance spectra's obtained for increasing concentrations of S. aureus (ATCC25923) from 10 to 10^7 cfu/ml in PBS and Fe(CN_6). (B) Equivalent electrical circuit used to the fit the impedance spectra.

The data of electrochemical impedance measurements can be fitted perfectly using the software Zplot/Zview. The equivalent circuit consists of the ohmic resistance (R_s), the charge transfer resistance (R_{CT}) and the constant phase element (CPE), figure 4 (B). A linear relationship between the polarization resistance and decimal logarithmic value of *S. aureus* concentrations was found ranging from 10 to 10^7 CFU/mL, with a slope of 14.66 and a correlation coefficient of 0.994 (figure 5).

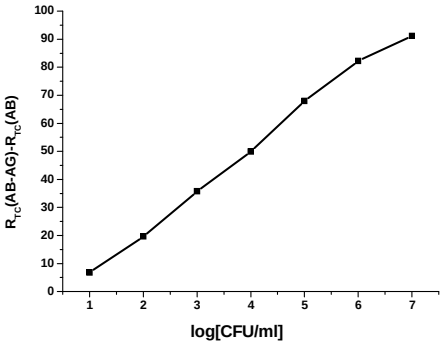

Fig. 5. Variation of the charge transfer resistance RTC with the logarithmic concentration of S. aureus from 10 to 10^7 cfu/ml. Values are normalized to R_{CT} in absence of bacteria detection, the datafollow a sigmoidal curve (fitr^2=0.994).

Conclusion

A simple and highly sensitive impedance based immunosensor was developed for detection of *S. aureus* bacteria. In fact, this work has demonstrated that the 16-Mercaptohexadecanoic acid, with a long alkyl chain and function group of carboxylic acid, has been successfuly deposited on the gold electrode, enabling the subsequent immobilisation of anti-S. aureus.

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