Biosensors and Bioelectronics 85 (2016) 103–109

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Inexpensive and fast pathogenic bacteria screening using field-effect transistors

Nello Formisano [a], Nikhil Bhalla [a], Mel Heeran [b], Juana Reyes Martinez [b], Amrita Sarkar [b], Maisem Laabei [c], Pawan Jolly [a], Chris R. Bowen [d], John T. Taylor [a], Sabine Flitsch [b], Pedro Estrela [a,*]

[a] Department of Electronic & Electrical Engineering, University of Bath, Bath BA2 7AY, United Kingdom
[b] School of Chemistry & Manchester Institute of Biotechnology, The University of Manchester, Manchester M1 7DN, United Kingdom
[c] Department of Biology & Biochemistry, University of Bath, Bath BA2 7AY, United Kingdom
[d] Department of Mechanical Engineering, University of Bath, Bath BA2 7AY, United Kingdom

ARTICLE INFO

Article history:
Received 25 December 2015
Received in revised form
3 April 2016
Accepted 20 April 2016
Available online 21 April 2016

Keywords:
Biosensors
BioFET
Electrochemical impedance spectroscopy
MALDI-ToF
Bacteria

ABSTRACT

While pathogenic bacteria contribute to a large number of globally important diseases and infections, current clinical diagnosis is based on processes that often involve culturing which can be time-consuming. Therefore, innovative, simple, rapid and low-cost solutions to effectively reduce the burden of bacterial infections are urgently needed. Here we demonstrate a label-free sensor for fast bacterial detection based on metal–oxide–semiconductor field-effect transistors (MOSFETs). The electric charge of bacteria binding to the glycosylated gates of a MOSFET enables quantification in a straightforward manner. We show that the limit of quantitation is 1.9×10^5 CFU/mL with this simple device, which is more than 10,000-times lower than is achieved with electrochemical impedance spectroscopy (EIS) and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-ToF) on the same modified surfaces. Moreover, the measurements are extremely fast and the sensor can be mass produced at trivial cost as a tool for initial screening of pathogens.

1. Introduction

Bacterial infection has a profound impact on global health (Coates et al., 2002) and contributes to globally important diseases, such as tuberculosis, pneumonia, tetanus, typhoid fever, diphtheria, syphilis, and is the cause of high infant mortality rates in developing countries; bacterial infection is also believed to be responsible for more than 20% of human tumours worldwide (Brachman and Abrutyn, 2009; Stein, 2011). The methods by which bacteria are currently detected in routine clinical microbiology involve processes consisting of an initial sample growth step where all the species in a sample are cultured in a rich medium in order to obtain a sufficient mass for a subsequent analysis. The post-growth analysis allows isolation and characterisation of single species in a sample, usually by means of techniques such as staining, real-time polymerase chain reaction (RT-PCR), whole genome sequencing (WGS) and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-ToF MS) (see *e.g.* Fournier et al., 2013 and references therein). Although elegant and effective alternative approaches to microorganism detection have been reported (Mannoor et al., 2012; Zourob et al., 2008; Ma et al., 2015), where single bacteria sensing could be achieved (Kang et al., 2014; Mohanty and Berry, 2008), the current 'gold standard' for clinicians is still represented by culturing methods, nucleic acid-based sensors, immunoassays and fluorescence-based techniques (Ahmed et al., 2014). Limitations on the adoption of new solutions for diagnosis include the complexity of the fabrication of new sensors, the complexity of assay implementation and sample processing, and the prohibitive costs of introducing new equipment to perform bacterial detection. In order to overcome such obstacles while simultaneously introducing improvements in the current diagnostics, simple, readily available sensors must be developed. The introduction of fast, simple and low-cost sensors that could be easily employed in clinical laboratories would dramatically reduce both the time and the cost of current bacterial diagnosis. For instance, a device that is

* Correspondence to: Department of Electronic & Electrical Engineering, University of Bath, Claverton Down, Bath BA2 7AY, United Kingdom.
E-mail addresses: n.f@hotmail.it (N. Formisano), nikhilbhalla151@gmail.com (N. Bhalla), juana.reyesmartinez@manchester.ac.uk (J. Reyes Martinez), amrita.sarkar@manchester.ac.uk (A. Sarkar), maisemlaabei27@gmail.com (M. Laabei), p.jolly@bath.ac.uk (P. Jolly), c.r.bowen@bath.ac.uk (C.R. Bowen), j.t.taylor@bath.ac.uk (J.T. Taylor), sabine.flitsch@manchester.ac.uk (S. Flitsch), p.estrela@bath.ac.uk (P. Estrela).

http://dx.doi.org/10.1016/j.bios.2016.04.063
0956-5663/

able to provide an initial screening of a sample at the initial growth stage with adequate detection regarding its pathogenicity would be able to confirm the need to perform more complex and expensive analysis. Given the status of national health systems worldwide and the ever present need to reduce the cost of public healthcare, only very inexpensive, easily fabricated devices that are able to perform in a rapid manner and with parallel screening would be currently capable of supporting effectively clinical microbiology.

We here report on a label-free bacterial detection system using biologically-sensitive field-effect transistors (BioFETs) (Poghossian and Schöning, 2014). The BioFETs were constructed by immobilising a bioreceptor layer onto an extended gate of a metal–oxide–semiconductor field-effect transistor (MOSFET). MOSFETs are ubiquitous electronic components that can easily and cheaply be expanded into arrays for high-throughput screening. BioFETs with extended gold gates have previously been used for the detection of DNA hybridisation (Estrela et al., 2005) and proteins (Estrela et al., 2010). In this study we demonstrate the detection of mannose-specific type 1 fimbrial *Escherichia coli* PKL1162 as a case study for MOSFET-based bacterial detection.

Amongst pathogenic bacteria, uropathogenic *Escherichia coli* (UPEC) are responsible for urinary tract infections. UPEC is the most predominant uropathogen causing approximately 80% of uncomplicated infections (Ronald, 2003). It is estimated that between 40 and 50% of females and 15% of males will develop urinary infections and the rate of recurrent infections has been reported to be as high as 30% (Foxman et al., 2000). In order to colonise the cells of the urinary conducts and trigger a disease, UPEC can exploit hair-like protein structures expressed on their surface (called fimbriae), which allow bacteria to firmly adhere on the cells' surface and not be washed away in the urinary flow. Lectin protein structures, that constitute fimbriae, are expressed in at least 9 out of 10 UPEC strains (Oelschlaeger et al., 2002). As the specificity can vary towards different glycosylated surfaces, several carbohydrate-specific fimbriae have been found and, amongst them, mannose-specific type 1 fimbriae is classified as one of the most commonly expressed (Hartmann and Lindhorst, 2011). The recognition event interests the glycosylated cell of the urinary tract and the mannose-specific protein, called FimH, situated at the tip of the fimbrial rod of the pathogenic bacteria.

The electric charge of bacteria binding to the glycosylated gate of a MOSFET enables quantification in a straightforward manner. Both the charges on the membrane of the bacteria and the displacement of water and ions from the biolayer surface when a bacteria is present, disturbs significantly the electrochemical double layer capacitance, which causes a threshold potential shift on the BioFETs. Very low limits of detection can be obtained with the technique. As a comparison, the same electrodes were used for electrochemical impedance spectroscopy (EIS) and matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-ToF) – both techniques show significantly higher limits of detection than the BioFETs.

2. Materials and methods

2.1. Bacteria preparation

Uropathogenic *Escherichia coli* strain PKL1162 was obtained by engineering the strain SAR18 with the plasmid pPKL174 (Reisner et al., 2003). The bacteria were grown overnight while shaking in an incubator at 37 °C in lysogen broth growing media. The bacteria were then harvested by centrifugation at 4000 rpm for 15 min at 4 °C. The pelleted bacterial cells were resuspended in 10 mL of PBS Buffer and harvested twice before a final resuspension in 1 mL of PBS Buffer for their quantification and use.

2.2. Electrode functionalisation and affinity capture assay

A mixture of HS-$(CH_2)_{17}$-$(OC_2H_4)_3$-OH and HS-$(CH_2)_{17}$-$(OC_2H_4)_6$-OCH_2COOH in a ratio 1:4 was used for the SAM formation. The mixture, obtained by mixing 53 µL of HS-$(CH_2)_{17}$-$(OC_2H_4)_6$-OCH_2COOH (0.476 mM, 0.2 mg/mL in DMSO) and 147 µL of HS-$(CH_2)_{17}$-$(OC_2H_4)_3$-OH (0.328 mM, 0.2 mg/mL in DMSO) was sonicated for 20 min and used for the overnight incubation with the electrodes at room temperature in a humidity chamber.

Aminoethyl glycosides in PBS at the concentration of 50 mM were immobilised overnight in humidity chamber at room temperature overnight after activation of the carboxyl groups using a solution of EDC/sulfo-NHS at the concentration of 40 mM and 10 mM, respectively, for 1 h. Once the glycosides were immobilised, a blocking step of the non-reacted sites was performed using a 10 mM ethanolamine aqueous solution at pH 8.5 for 20 min. The bacteria affinity capture was performed by incubating the electrodes at 37 °C for 1.5 h.

Immobilised α-D-mannose was used to affinity capture uropathogenic *Escherichia coli* strain PKL1162 while 2-Acetamido-2-deoxy-α-D-galactopyranose (GalNAc) was used as a control glycan. A further control was performed by measuring the interaction between α-D-mannose and the *Escherichia coli* strain K12.

The assay was carried out on in-house fabricated arrays of gold electrodes (100 nm thickness deposited on 20 nm of chromium on glass substrates by means of thermal evaporation through a shadow mask).

2.3. Measurements set up

The extended-gate FET sensor with gold thin film electrode consisted of two parts: (i) an array of gold electrodes, where bacteria were captured, and (ii) the FET structure, which transduces the binding events on the gold electrode into electrical signals. The extended gate was fabricated by connecting the Au electrodes, fixed in a reaction cell, to the gate of a MOSFET *via* a metal wire.

The BioFET measurements were carried out connecting the in-house fabricated arrays of gold electrodes to the gate of an n-type MOSFET. The MOSFET readings were taken using an Agilent B1500A HR CMU Semiconductor Device Analyser.

The bacterial detection is initially demonstrated by performing electrochemical impedance spectroscopy (EIS) capacitive measurements. The capacitance (C) indicates the capacity of a material to store charge (Q) due to a potential difference (V) and is given by the expression $C = \frac{Q}{V}$, which, for a parallel plate capacitor, can be rewritten as $C = \frac{\varepsilon_0 \varepsilon_r A}{d}$, where ε_0 is the permittivity of the vacuum, ε_r is the relative permittivity that depends on the material between the two plates having a surface area A that are a distance d from each other. The imaginary and real part of the complex capacitance (C' and C'' respectively) were calculated from the measured impedance using Eq. (1) (Formisano et al., 2015):

$$C^* = C' + jC'' = \frac{1}{j\omega Z} \quad (1)$$

The percentage change of capacitance from each step where then calculated considering only the real part of the capacitance, C', at the frequency where the absolute value of the imaginary value, C'', has its relative minimum. This frequency was 10 Hz throughout the experiments.

For the EIS recordings, non-Faradaic measurements were carried out in a phosphate buffer saline (PBS) solution diluted 100