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T. Raberalam, M. Balde, S. Hantova, A. Vena, H. Marchandin, et al.. Feasibility of Capacitive Aptasensor on Paper to Detect Pathogenic Bacteria Responsible for Nosocomial Infection. *Procedia Technology*, 2017, 27, pp.295 - 298. 10.1016/j.protcy.2017.04.122 . hal-01628284

HAL Id: hal-01628284

<https://hal.science/hal-01628284>

Submitted on 16 Dec 2021

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Biosensors 2016

Feasibility of capacitive aptasensor on paper to detect pathogenic bacteria responsible for nosocomial infection

T. Raberalam^a, M. Balde^a, S. Hantova^b, A. Vena^a, H. Marchandin^b, B. Sorli^a *

^aUMR 5214 Institut Electronique et des systemes, Université de Montpellier, 34000 Montpellier, France

^bUMR 5569, Hydrosiences, Université de Montpellier, 34000 Montpellier, France

Abstract

Nosocomial infections are contracted in a hospital or health care facility. Bacterial cause is the most common in 87% of cases. Studies have been conducted for the detection of these bacteria. The aim of this biosensor is to make an early detection tool, easy to use, reliable, not invasive and low cost. The goal is to detect in real time and simultaneously in a small amount of body fluids such as urine, sweat or saliva, the major bacterial pathogens causing these diseases: *Escherichia Coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus* and *Clostridium difficile*.

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Peer-review under responsibility of the organizing committee of Biosensors 2016

Keywords: Biosensor – POC Test – Sensor on flexible substrate

* Corresponding author. Tel.: +0-000-000-0000 ; fax: +0-000-000-0000 .
E-mail address: brice.sorli@ies.univ-montp2.fr

1. Introduction

In the world, the average is 5 to 10% of hospitalized patients are affected by nosocomial infections [1, 2]. These infections are contracted in a hospital or health care facility. They occur after 48 hours following hospitalization of medical care. In 87% cases, bacteria are the most common cause. Besides that, infected patients are usually asymptomatic in the early stages of infection. Because of this, many health complications appear mainly due to a diagnostic delay. The detection and rapid identification of these bacteria are particularly useful. However, the vast majority of healthcare facilities is affected by nosocomial infections, so medical fields need new tools of bacteria analysis, so medical fields need new tools of bacteria analysis, more fast, cheapest, efficient and reliable.

Our biosensor is to make an early detection tool, easy to use, reliable, not invasive and low cost. The goal is to detect in real time and simultaneously in a small amount of body fluids such as urine, sweat or saliva, 5 of the major bacterial pathogens causing these diseases (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Clostridium difficile*). First, we based on a feasibility study for the detection of one of these pathogenic bacteria: *Escherichia Coli*.

2. Materials and methods

2.1. Support

The manufacture of the support is made within the laboratory. The support consists of a flexible substrate and a thin aluminum deposited by electro-evaporation layer, and then oxidized by anodization process to form a nanoporous alumina layer. The porous diameter can be variable according to the needs. For this biosensor, porous diameter is around 80 nm to 300 nm [3]. They have interesting properties as biocompatibility, thermal stability and they confer a good adhesion to ligand.

2.2. Targets and Ligands

Our mains targets are membrane proteins (Lipopolysaccharide – LPS / Peptidoglycan -PPG), mainly present on the outer membrane of bacteria. Their presence even in small doses can cause diseases. And we used specific aptamers as ligands (DNA). They are specific and have good affinity to their targets, stable in the long term, low cost production, their properties can be adapted to the request.

2.3. Assembly process

When the support is ready to use, we perform a functionalization on the support by the SAM Method (Self Assembled Monolayer) [4]. Then, we have molecular assemblies (organic molecule) formed spontaneously on the support by adsorption. From there, we immobilized the specific aptamers. The vast majority of the support is coated and functionalization rate is around 90%. An interdigital electrode (AI) is filed on the top of the functionalized support to allow the measurement.

2.4. Measure

We use bacterial culture to make the measurements. We make serial dilutions for having increasing concentrations of bacterial solution. To save the electric variations of the biosensor, we use Impedancemeter HP4053 with Labview software. This allows us to follow the capacitance measurement versus the time.

2.5. Results

Bacteria in the sample will bind to their specific aptamers. Aptamers change structure and they will lead an electrically variable on the support

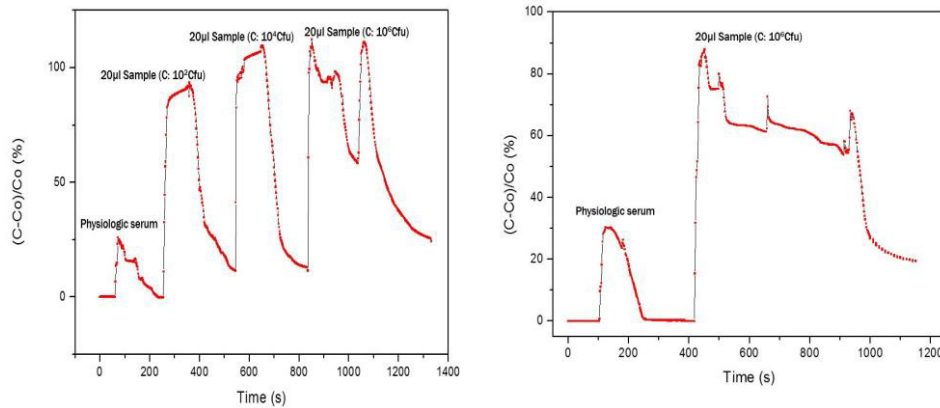


Figure: Capacitance measurement versus the time

- First we file 20µl of physiologic serum at the center of the interdigital electrode. We start the capacitive measurement. After, we put at the same location, 20µl of sample - C: 10⁶Cfu. The measurement is stopped after drying and stabilization of resulting values
- For the second curve, we put 3 concentrations of sample (10² Cfu -10⁴ Cfu -10⁶Cfu) at the same quantity (20µl). The process is the same and we are waiting after the last stabilization of values to stop the measurement.

3. Conclusion

For this biosensor on paper, we can deduce that the presence of bacteria induce an electrical variation. After counting, we can detect around 390 bacteria in 20µl of sample, it's relatively sensitive. We associate the settling time to the bacterial lifetime, because when the deposited sample is dry, values are beginning to stabilize. Bacteria can only live on their nutrient medium (here, physiological serum), and when all the liquid evaporates, bacteria are

immobilized on the surface of the support. And they probably die. It's a point on which we are working. We are also working on RFID and NFC transmission to pair with the biosensor.

The main specifications of this biosensor are: Easy handling, thermally stable, non-invasive, fast and specific detection, low cost due to his fabrication process, doesn't require a treatment of the sample. And future applications / embodiments that we envisage for this biosensor are: Connected wound dressing, applied in hospitals and healthcare facilities for monitoring and control of bacterial infections. But we can also think about adapting it to other areas such as the environment or food.

Acknowledgements

Authors thank the "LABEX NUMEV" for funding this study.

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