CrossMark

Label-free interdigitated microelectrode based biosensors for bacterial biofilm growth monitoring using Petri dishes

Jacobo Paredes *, Sheila Becerro, Sergio Arana

CEIT and Tecnun (University of Navarra), Paseo de Manuel Lardizábal, n° 15, 20018 Donostia-San Sebastián, Spain
CIC microGUNE, Goiru Kalea 9, 20500 Arrasate-Mondragon, Spain

ABSTRACT

Impedance microbiology (IM) is a known technique that has been applied during the last decades to detect the presence of microorganisms in real samples in different fields: food industry, healthcare, environment, etc. Bacterial biofilms however have not been so far studied despite the fact that they are the most common microbiological formation and that they present resistance to antimicrobial agents. In situ early detection of bacterial biofilm is still a challenge nowadays that causes huge impact in many different scenarios. The ability to detect biofilm generation early will allow better and more efficient treatments preventing high costs and important problems. In this work a new performance of this technique with interdigitated microelectrode sensors (IDE) is proposed. A specific culturing setup where the sensors have been integrated in Petri Dishes has been developed. From the results it can be highlighted that low frequencies are more sensitive for detection than higher ones. The results achieved record variations of approximately 40% in the equivalent serial resistance after 10 h of culture. Electrical models have been successfully simulated to find the electrical behavior of the development of biofilms. Variations in both the capacitance and resistance were recorded during the growth of the microbes.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Bacterial biofilms are complex biological structures spontaneously formed by microorganisms in its normal growing behavior. These living systems are composed of bacteria and a polymeric extracellular matrix that houses the microbes providing a unique environment. The generation of the biofilm starts with an irreversible attachment of one or more bacteria to a preferably inert surface in a nutrient environment. Then a sequential cascade of biochemical signaling, involving quorum sensing, genomic mutations, etc. starts promoting changes in the cells. The more visible effect lies on the production of the polysaccharide-based slime that houses the microbes (Blenkinsopp and Costerton, 1991).

Bacteria present high resistance to antimicrobial agents once the biofilm reaches the mature stage, making their elimination a complex problem (Hall-Stoodley et al., 2004; Davies, 2003). It has been described that biofilm bacterial cells present from 500 to 5000 times higher resistance to antibiotics than non-biofilm bacteria (del Pozo et al., 2009). However microorganisms are still susceptible to the action of antimicrobial agents at the early stages of the development of the biofilm.

There are many novel strategies that involve preventive actions to break up the biofilm resistance to antimicrobial agents. Most of them are based on different protocols that seek to weaken the polysaccharide slime (Høiby et al., 2010; Stewart and William Costerton, 2001). However these approaches are not always effective, and so leading in an increment of biofilm coating or resistance (Simões et al., 2010). Early detection techniques are the alternative to increase the effectiveness of antimicrobial agents in a shorter time (Ehrlich et al., 2005).

Biofilms are as well as bacteria ubiquitous in the nature so they are present in a vast number of different environments. Healthcare environment (indwelling medical implants as most important focus) is one of the most relevant fields where the presence of biofilms causes serious damages. Biofilm-related infections are a real health problem, not only because of the pathology but also because the antibiotic treatments are not being effective in killing the microbes (del Pozo and Patel, 2007). Food industry is also another important field where a high nutrient environment favors the development of foodborne pathogens contaminating the products. Obviously, this causes high risk and impact on population's healthcare (Shi and Zhu, 2009). Industrial environments, water exchangers, pipes lines, etc. are also exposed to a decrease on its efficiency or even to the contamination of the contents (Donlan, 2002).

Traditional analysis methods normally involve culturing the samples in agar plates and some complementary DNA assay or immuno analysis. All this process takes often too much time delaying important clinical decisions. It is suggested in bibliography that small devices based on microfluidics will reduce the time analysis to the order of 2 to 4 h with a more accurate specific recognition of the biological targets (Radke and Alocilja, 2004).

* Corresponding author at: CEIT and Tecnun (University of Navarra), Paseo de Manuel Lardizábal, n° 15, 20018 Donostia-San Sebastián, Spain. Tel.: +34 943 212 800; fax: +34 943 213 076.

E-mail address: jparedes@tecnun.es (J. Paredes).

Electrical impedance of biofilms is a specific parameter to identify the presence of these biological systems (Varshney and Li, 2009). A large number of the works found in bibliography use different binding elements for specific biorecognition at the single bacteria level. However most of these works are focused on devices designed for a one single time analysis. There are also other works that detect bacteria based on measurements of the metabolic activity of bacterial communities (Gómez et al., 2002). These detecting approaches are known as indirect detection methods. Usually these methods allow the monitoring of bacterial growth in the culturing vessel. But above all those, label-free and surface-free sensors are suitable for long term applications by monitoring the sample and detecting the growth kinetics curves of the microbes (Tang et al., 2011).

In this work label-free interdigitated microelectrodes (IDE) are used for in vitro growth monitoring of biofilms in Petri dishes (PD). Real time characterization of the impedimetric behavior of microbiological biofilms is performed, showing that this technique is an effective way to detect the presence and the growth of bacterial biofilms. Moreover, the discussion about the sensitivity of different electrical parameters is presented. From the conclusions of this research it can be said that potentially this kind of sensors can be applied to a wide variety of problems where in situ and early detection is required for preventing further problems.

2. Materials and methods

2.1. Chemicals and reagents

The culturing media used in this work were Tryptic Soy Broth, TSB (BBL™, ref: 211768) enriched with 5% glucose (Dextrose from Difco™, ref: 215530). All solutions and media were prepared with deionized water (Merck Millipore) and media were also sterilized at 121 °C for 1 h in the autoclave. Ethanol at 99.5% (Panreac ref: 131659) and 10% Hellmanex II dissolution (Hellma Analytics ref: 9-307-010-507) were used for cleaning biosensors.

2.2. Microorganisms and culture protocol

Bacterial cultures were performed following the protocol described in previous works (Paredes et al., 2013). *Staphylococcus epidermidis* (SE) (ATCC 35984) was used to prepare a 0.5 McFarland suspension to later infect the culture media. This strain was purchased from the Colección Española de Cultivos Tipo (CECT) and stored frozen at -80 °C in a Cryoinstant® mixed vial (pH 7.2 $\pm$ 0.2 obtained from Scharlau ref: 064-TA8276). It was reviewed as a high biofilm trend strain suitable for this work.

Inoculum concentration was calculated counting the number of Colony Forming Units on culturing plates after plating and harvesting 100 μL of sample.

2.3. Experimental setup and biosensor

Fig. 1 shows a CAD image of the measurement and biofilm culture system which uses Petri dishes as main structure. The design of the IDE based sensor can be observed in Fig. 1(a). A real photography of an assembled chip holder with some biosensors connected to a connection board is shown in Fig. 1(c). In a previous work, this chip holder was applied to 96-well plates (Paredes et al., 2013), where the volume was considerably lower. Instead of having 8 different vessels, there is only one vessel in which the biosensors are submerged providing a more uniform assay. Moreover, this setup provides better statistical and consistent results. Up to eight sensors can be analyzed in the same culturing conditions providing the necessary number of samples to discard malfunctions and other effects. The versatility of the chip holders allows holding different sensors (varying geometries and/or materials) that can potentially highlight the key parameters to improve the sensitivity.
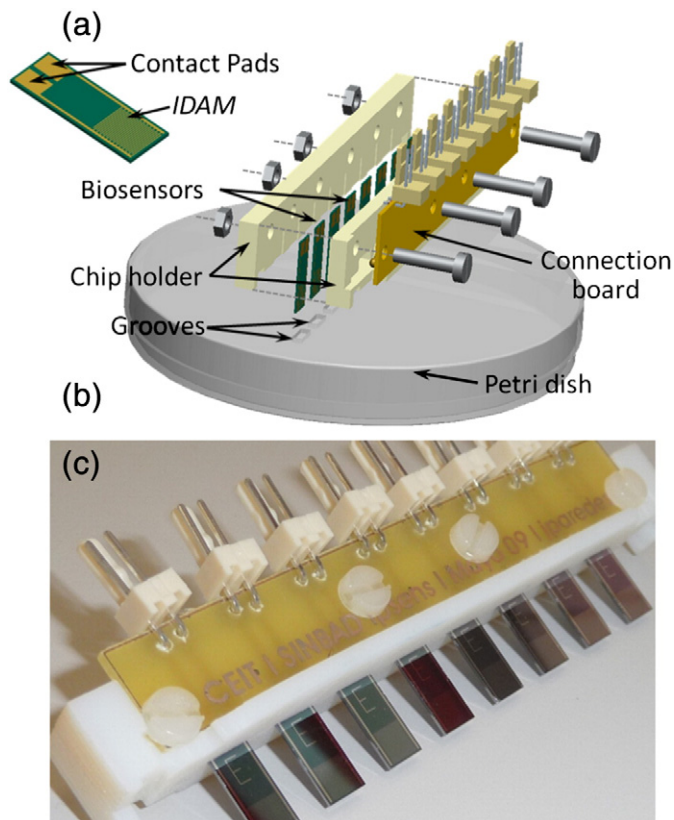

Fig. 1. Experimental setup for electrical impedance measurements with an IDE based sensor. (a) Detail of the biosensor with its connection paths and an array of 40 pairs of fingers, (b) CAD image of the measurement and culture system and (c) real photograph of an assembled chip holder ready for its utilization in impedimetric measurements.

If necessary, a number of PD could be used in parallel with different experimental conditions.

There are some alternative setups that have been reported in bibliography, which basically use microfluidics to provide an access to a culturing chamber where a sensor is placed (Varshney and Li, 2008; Gomez-Sjoberg et al., 2005). These kinds of setups could be as well applied to a variety of cellular cultures, from bacterial to mammalian cells (Wegener et al., 2000). Many of these studies are focused on monitoring cellular attachment, growth, or response to certain treatments (Brischwein et al., 2003; Otto et al., 2004).

Petri dishes are widely used, not only for microbiology experiments but also for mammalian cellular cultures. One of the most important aspects of this new setup is the relatively low fabrication cost. Nonetheless, this new configuration provides a reliable culturing system that allows monitoring impedance variations of the cultures in real time. The vertical disposition of the biosensors is going to be discussed in the following sections.

2.4. Biosensors and impedance measurements

The sensors used in impedance spectroscopy have been designed and fabricated by means of silicon microtechnologies on 3 in. wafers. The IDE biosensors consist of a 100 nm thin film layer of gold sputtered onto a 15 nm chromium adhesion layer deposited onto the thermally oxidized silicon wafer. Each array has an active area of 16 mm² formed by 40 pairs of micro electrodes. The finger length is 3980 μm, the electrode width 20 μm and the electrode gap 30 μm. The final dimensions of the sensors are 5 mm width and 15 mm length.

Before using them for impedance measurements, sensors have been cleaned during 10 min with Hellmanex® cleaning solution in an ultrasonic bath. Next, the same step is repeated using Milli-Q® water in