MONITORING BIOFILM GROWTH USING A SCALABLE MULTICHANNEL IMPEDIMETRIC BIOSENSOR

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ABSTRACT

In this work we report on a multichannel electrochemical impedance spectroscopy (EIS)-based biosensor that allows the monitoring of biofilm growth. Moreover this approach is highly scalable and can easily be integrated into a microfluidic flow cell. Two bacterial strains (pseudomonas aeruginosa, stenotrophomonas maltophilia) were successfully monitored for up to 96 hours without causing biosensor saturation.

KEYWORDS: Impedimetric biosensor, Biofilm growth, Electrochemical impedance spectroscopy, Microfluidic

INTRODUCTION

Bacterial film growth on solid surfaces has increasingly gained more attention during the past decades because of its wide range of applications; such as industrial, maritime, medical and environmental. There is a number of analytical methods available for the characterization of a biofilm, the most commonly used techniques include gravimetric methods such as quartz crystal microbalances or surface acoustic waves [1], optical microscopy, surface plasmon resonance (SPR) as well as electrochemical methods. The piezoelectric-based and the SPR-based systems show a high sensitivity but lack the capacity to scale up while remaining low-cost. Optical methods present a low-cost analysis but are disruptive and can not carry out an online monitoring of the biofilm growth. Electrochemical impedance spectroscopy (EIS) has recently been studied as an alternative method [2, 3] which is a powerful, non disruptive and online method to measure the electrical properties of materials.

THEORY

EIS is based on the monitoring of the resistance and reactance of a surface by means of applying a small-amplitude AC signal with variable frequency through a pair of electrodes and measuring the resulting current (see Figure 1a). The real and imaginary parts (i.e., resistance and reactance) of the impedance are plotted as a function of frequency in a Nyquist diagram (see Figure 1b). The biofilm growth will hinder the charge transfer between the electrodes and therefore increase the measured impedance. This change can clearly be seen in the Nyquist diagram as a curve shift in both axis.

EXPERIMENTAL

We have built a custom made EIS-based measurement cell consisting of a polymer microfluidic flow cell, gold sputtered electrodes on a Cyclic Olefin Copolymer (COC) substrate (see Figure 2) and a measurement electronics (see Figure 4b). Firstly the COC substrates were sputtered with a 100 nm thick gold layer followed by a positive photoresist deposition by spin coating. Subsequently the substrate is exposed to UV light through a mask which contains the electrode design; afterwards the photoresist is developed, leaving the undesired gold area exposed. Finally the gold is etched away with a KI/I₂ solution and the remaining photoresist is removed.
Figure 2: Electrodes manufacturing steps 1) COC substrate 2) Gold sputtering 3) Positive photoresist deposition 4) UV exposure through photomask 5) Exposed photoresist becomes soluble 6) Photoresist developing 7) Exposed gold is etched.

The fluidic device consists of PDMS flow cell, a PMMA upper frame and bottom plate and the COC substrate, which serves as the substrate for the electrodes and as the bottom part of the polymer PDMS channel (see Figure 3). The PDMS flow cell had 2 independent channels, one for the measurement and another for the reference electrodes both measuring 26 × 4 × 0.4 mm. Each flow chamber contained 4 differently shaped electrodes (see Figure 4a) in order to determine the shape influence in the sensitivity of the electrode.

Figure 3: a) Fluidic device schematic b) Flow channel in detail

The amplitude of the AC input voltage was 40 mV peak-to-peak and the frequency used to make the measurement was chosen to be 1100 Hz, it is a compromise between sensor sensitivity and drift. Using this setup we could successfully monitor the growth of two different bacteria (*Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*). During the measurement the respective bacteria was probed with an OD_{600} (optical density at 600 nm) of 0.5 across the electrodes for an interval of three hours followed by a continuous purge with feeding medium (Brain Heart Infusion diluted 1:4 with distilled H_{2}O).

Figure 4: a) One half of the custom gold electrode design and connection pins. The impedance is measured between the large counter electrode and the working electrode. b) Measurement setup consisting of: 1) flow cell with 2 separate channels, 2) custom made electronics.
RESULTS AND DISCUSSION

Analyzing the result in Figure 5 one can see that the impedance increased over time because of a biofilm on the electrode surface. In order to compensate drift effects (mainly due to ambient temperature changes) we used two electrodes: the measurement electrode was probed with the bacteria whilst the reference electrode was only exposed to feeding medium. The impedance was measured during the whole experiment for both electrodes. After the experiment the electrodes were inspected by means of microscopy in order to confirm the growth (in case of the measurement electrode) and the absence (in case of the reference electrode) of a biofilm (see Figure 5, inlet images). After twenty hours (three hours of probing with bacteria and seventeen hours of feeding medium) there was already a clear increase in the impedance of the measurement electrode which did not reach saturation during the whole 96-hours experiment.

CONCLUSION AND OUTLOOK

We present a biosensor that allows monitor of eight electrodes simultaneously divided into two separated microfluidic channels, one for the reference and the other for the measurement electrode. After 20 hours of experiment the results clearly showed an impedance increase of the measurement electrode, this trend continued throughout the whole 96 hours of the assay without showing saturation signs. Posterior microscopy analysis confirmed the biofilm growth on the measurement electrode and denied its presence on the reference electrode. We will show that this setup is able to monitor biofilm growth in 24 channels each equipped with 4 electrodes at a time. Such a system allows the rapid screening both of potential biofilm forming species as well as the effect of physical or chemical treatments in order to dissolve the formed biofilm.

REFERENCES


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