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Electrical detection and characterization of bacterial adhesion using electrochemical impedance spectroscopy-based flow chamber

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Abstract

In the present work, we report on the electrochemical detection and characterization of bacterial adhesion onto a semiconducting indium tin oxide (ITO) plate using an electrochemical impedance spectroscopy (EIS)-based flow chamber. We used two different bacterial strains (*Pseudomonas stutzeri* (PS) and *Staphylococcus epidermidis* (SE)) so that their adhesion behavior and charge transporting property could be compared. The electrical detection was achieved by monitoring the impedance variations in the low frequency range during the adhesion process of both bacterial strains. The electrical characterization was achieved by measuring the impedance over a large frequency range before and after 2 hr of adhesion. The electrical properties of the electrode/bacteria/electrolyte interfaces were explained in terms of resistances and capacitances of an equivalent circuit whose frequency-dependant impedance was fitted to the measured data curves. The magnitude of the impedance was found to decay exponentially as the number of adhering cells increased during the deposition time for both bacterial cells. The adhesion of PS bacteria was detected electrically before SE bacteria. Also, the impedance fitting results revealed that PS bacterial cell allow more charge transfer to the electrode than SE bacterial cell, and therefore it donates more charges and adheres faster and more firmly to ITO surface.

Keywords: Bacterial adhesion; Electrochemical impedance spectroscopy; Charge transfer; Flow chamber

1. Introduction

In recent decades, studying and understanding the mechanism of bacterial cell adhesion to solid surfaces is becoming increasingly important since it touches various domains of applications, such as biomedical, industrial, marine and environmental. Bacterial adhesion to the material surface is a complicated process that is affected by various physico-chemical properties of the bacteria cell and substratum surfaces, including the hydrophobicity, surface potential and charge as well as the dielectric and conducting properties [1–4]. Initial bacterial adhesion can be described by the so-called extended DLVO (Derjaguin, Landau, Verwey, Overbeek) theory in which adhesion is predicted as an interplay of Lifshitz–van der Waals interactions, electrostatic interactions and Lewis acid–base interactions between the interacting surfaces [5,6]. In this theory and when analysing electrostatic interactions, the potential of the interacting surfaces is usually assumed to be constant. However, both the surface potential as well as the surface charge density may change during adhesion due to charge transfer between the interacting surfaces [7].

Charge transfer between bacteria and conducting or semiconducting material surfaces plays an important role in initial bacterial adhesion. In conducting (or semi-conducting) materials, free electrons are present which give rise to short-range electron exchange interactions [8]. In addition, bacterial cell surface consists of a variety of different macromolecules including proteins, which contain electrochemical active groups; particularly carboxylate functions that facilitate charge transfer and/or charge exchange [9]. As a result the bacterial cell surface possesses free electrons, which might be exchanged with a conducting surface during initial adhesion. The exchange of charge can be a charge transfer either to or from the bacterium sur-

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face, depending on the surface potential and ionic strength of the suspending medium [7]. In fact, charge transfer at the bacteria/substrate interface could be measured during initial adhesion. Poortinga et al. [7] used a parallel plate flow chamber to simultaneously measure bacterial adhesion to the semiconducting indium tin oxide (ITO) surface and the change in both the electric potential and capacitance of the surface during adhesion. They concluded that on average a charge of about 10^{-14} C per bacterium is transfered during initial adhesion, which corresponds to a small fraction of the total surface charge of a bacterium. Also, it has been shown that charge transfer depends on the specific resistivity of the semiconducting titanium-oxy-nitride substrata during bacterial adhesion, with an influence on the strength of adhesion [9]. The dependence of bacterial adhesion on the electrical potential of the substratum has been shown as well by Busalmen and de Sánchez [10]. Using an electrochemical thin-film flow cell, they demonstrated that bacterial adhesion was irreversible on thin films of gold of positive potential, while it was reversible and strongly inhibited on polarized thin films of gold of negative potentials. Also, Poortinga et al. have shown that bacteria reversibly adhered on ITO electrode surface could be desorbed by applying a negative potential at the electrode. However, bacteria irreversibly adhered on a high positive potential electrode could hardly be desorbed, by applying negative potential, from the electrode surface [11].

It is generally believed that charge transfer is a fundamental process in electrochemical techniques and also an omnipresent process in biological and chemical systems [12]. Since charge transfer is a process involved in bacterial cell adhesion, electrochemical impedance spectroscopy (EIS) could be used in the investigation of bacterial adhesion on conducting or semiconducting surfaces. EIS technique has emerged as a promising analytical tool for the development of sensor devices and for the investigation of bulk and interfacial electrical changes on an electrode surface used as a transducer [13]. Therefore, any intrinsic electrical property of adhering bacteria cells onto the electrode surface that could affect the conductivity in the electrochemical process can be potentially detected using this technique.

EIS technique has been applied in the development of biosensors in order to detect bacterial cells and their growth either in suspension or immobilized on a modified electrode. For example, a three-electrode electrochemical cell was used to electrochemically detect the growth of viable *Salmonella typhimurium* in high and low frequency ranges [14]. The adsorption of bacteria on the electrode surface could affect dramatically the variations of the real and imaginary impedance components at a detection time. In another work, Yang et al. developed ITO interdigitated microelectrodes used as impedance sensors for rapid detection of *S. typhimurium* in a selective medium and milk samples [15]. Their results showed that bacterial attachment on the electrode increased the double-layer capacitance at low frequency and caused a consequent decrease in impedance. Similarly, Yang and Li used an interdigitated microelectrode coupled with immunomagnetic beads to detect selectively *S. typhimurium* and measure the changes in the double-layer capacitance of the electrode during the growth of suspended bacteria [16]. In addition, they used impedance spectroscopy to characterize electrically the immobilization of antibodies on ITO electrode through a self-assembled monolayer (SAM) of epoxysilane and the detection of *Escherichia coli* bacteria [17]. The immobilization of these bacteria has increased the charge transfer resistance more significantly than other electrical components. In a similar report, Chen et al. detected and characterized the immobilization of yeast cells on a self-assembled monolayer of alkanethiolate using impedance spectroscopy [18]. They showed that SAM assembly and subsequent yeast immobilization greatly increased the electron transfer resistance and decreased the double-layer capacitance.

Recently, Boehm et al. developed a simple and rapid method for the detection and identification of bacteria using a microfluidic testing chamber. The microfluidic chip utilizes impedance-based measurement to detect cells and identify them when used in conjunction with immobilized monoclonal antibodies [19]. Similarly, Varshney et al. developed an impedance biosensor to rapidly detect pathogenic bacteria in ground beef samples. The biosensor consisted of a microfluidic flow cell with embedded gold interdigitated array microelectrode [20].

However, all of these published works are focused on the detection of the adsorption or attachment of suspended bacteria cells or immobilization of bacterial cells on modified electrode surface, but not on the adhesion kinetics. Also, the disadvantage of using a conventional three-electrode electrochemical cell is that there is no microscopic control on the kinetics of the adsorption or attachment process of suspended bacterial cells during the impedance spectroscopy measurements. It was pointed out that a reliable study of bacterial attachment requires well-defined hydrodynamic conditions [21]. Parallel plate flow chamber has turned out to be an effective tool and has been widely used by many researchers to study properly the adhesion kinetic of different bacterial species on many types of materials [21–23]. The advantage of using this type of chamber is that one can study the adhesion kinetics under controlled hydrodynamic conditions and microscopic observations. Therefore, combining the hydrodynamic method with EIS technique was necessary in this study. To the best of author's knowledge, this is the first time in which the bacterial adhesion is detected and characterized using EIS-based flow chamber technique.

The purpose of this study was to detect and characterize electrically the adhesion of two different bacterial strains (*Pseudomonas stutzeri* (PS), and *Staphylococcus epidermidis* (SE)) using the electrochemical technique based on the charge transfer process involved in the adhesion mechanism between the bacterial cell and ITO semiconducting surfaces. An EIS-based flow chamber combining a controlled hydrodynamic system and the electrochemical impedance spectroscopy was then developed and used simultaneously for bacterial deposition and impedance spectroscopy measurements. For both bacterial strains, the electrical detection was achieved by monitoring the impedance variations in the low frequency range during adhesion process, while the electrical characterization was achieved by measuring the impedance over a large-frequency range before and after bacterial adhesion. The electrical properties of the interface electrode/bacteria/electrolyte were explained in terms of