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An impedimetric sensor for monitoring the growth of *Staphylococcus epidermidis*

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Abstract— **There is a need for accurate, reliable methods of detecting bacteria for a range of applications. One organism that is commonly found in urinary catheter infections is *Staphylococcus epidermidis*. Current methods to determine the presence of an infection require the removal of catheters. An alternative approach may be the use of *in vivo* sensing for bacterial/biofilm detection. This work investigates electrical impedance spectroscopy to detect the growth of *Staphylococcus epidermidis* RP62A on gold electrodes fabricated on a flexible substrate. Impedance spectra measured during biofilm formation on the electrode surface showed an increase in charge transfer resistance (R_{CT}) with time.**

I. INTRODUCTION

Impedance microbiology was first introduced over 100 years ago at the British Medical association in 1898 by Stewart.[1] Stewart monitored the purification of blood over a period of thirty days, reporting changes in the medium conductivity with the growth of bacteria. The principle upon which impedance microbiology is based was defined by Cady *et al* 1978.[2]

Most impedance measurements that adopt Cady's approach look for changes in the bulk resistance of the solution at only one frequency, usually with a two electrode technique. Some authors have focused on measuring bulk changes in the resistance of the growth media[2], by adjusting the constituents to maximise impedance changes [3, 4, 5]. However, the behaviour of the impedance response at the electrode-medium interface has been largely ignored. Felice *et al*[6] examined the medium and interface contributions during growth of *Escherichia coli* and compared two and four electrode techniques. They were one of few authors to present data using the complex plot and highlighted the need for more work on the interfacial response.

The objective of the current work was to monitor growth of *Staphylococcus epidermidis* RP62A on the surface of gold deposited on polyimide, using electrical impedance spectroscopy (EIS). *Staphylococcus epidermidis* is typically found on the human skin where it forms part of the natural skin flora. This organism, which is an opportunistic pathogen, will form a biofilm under certain environmental conditions and has been implicated as a major cause of hospital acquired infections, primarily urinary catheter infections. Electrical impedance spectroscopy differs from traditional impedance microbiology measurements in that it measures the impedance response over a wide range of frequencies and the data can be modelled using an equivalent circuit which can be interpreted in terms of the electrochemical reactions occurring within the system.

The results presented here demonstrate how EIS may be used as a non-destructive rapid method of examining the bulk and interfacial properties of the measurement cell during the growth of *Staphylococcus epidermidis* RP62A.

II. MATERIALS AND METHODS

Experiments monitored the electrical impedance spectra during the formation of the microbial biofilm *Staphylococcus epidermidis* RP62A, using a Solartron 1260 frequency response analyzer combined with a Solartron 1287 electrochemical interface. The preparation of the medium and culture together with the experimental protocol are presented below. All experiments were thermostatically controlled using an incubator at 37°C ± 0.1°C.

A. Microbiological Culture

All experiments were carried out using brain heart infusion (BHI) media (Oxoid), at 37 °C contained in sterile culture dishes. The media was prepared using the manufacturer's instructions and was sterilized before use by autoclaving. Culture dishes containing 2 ml sterile BHI media were inoculated with 200 µL of an overnight culture of *Staphylococcus epidermidis* RP62A. Electrodes were immersed in the sterile media two hours prior to inoculation.

A. Electrode Configuration

Electrodes were fabricated by deposition of gold on polyimide (PI). 125 µm Du Pont Kapton™ was plasma cleaned using helium plasma (60 W for 2 minutes). A 20 nm titanium inter-layer was sputter-deposited on the polyimide, followed by a 200 nm layer of gold. A series of photolithographic steps were used to form the electrode pattern shown in Fig. 1. An insulating layer of PI photoresist was applied to produce desired exposed electrode areas. The electrode array was designed so that it could be used for two and three-electrode techniques although only the results

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obtained using a two-electrode technique are presented here. The two-electrode technique, measured the impedance response between electrodes B and C (electrode A was not used for measurements). Electrodes B and C are identical in size (1 mm^2) and shape.

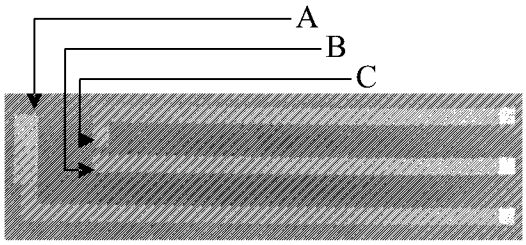

Fig. 1. Miniature EIS probes made by gold deposition onto polyimide.

Before impedance measurements the electrodes were cleaned with Piranha solution (H_2SO_4 : H_2O_2 3:1) then rinsed thoroughly with sterile distilled water and dried in N_2 atmosphere.

B. Impedance Method

Impedance spectroscopy was performed with the Solartron 1260 impedance/gain phase analyzer and the Solartron electrochemical interface 1287. An oscillating ac potential of 20 mV was applied to the system (0 V dc-bias) over the frequency range 10 Hz to 1 MHz. Impedance measurements were taken every 30 min for a period of 24 hours following inoculation of BHI media with *Staphylococcus epidermidis* RP62A. A control cell was setup containing sterile BHI media without bacteria and measurements were taken at the same intervals for comparison. All measurements were controlled using the Zplot software provided by Solartron Inc. The real and the imaginary parts of the impedance were displayed using the Zview component of the Zplot software. Complex impedance plots were modelled using the equivalent circuit provided by the Zview software (Fig. 2). Experimental values for changes in the bulk resistance R_s, the charge transfer resistance R_{CT} and the formation of the double layer capacitance C_{DL} are presented in section III.

D. The Equivalent Circuit Model

When an electrode is placed into an electrolyte a potential difference is established. Helmoltz proposed that the potential difference is created by two layers of charge with opposite polarity, with one layer located at the metal surface and the other of equal and opposite charge positioned just inside the electrolyte. This double layer has been termed the double layer capacitance C_{DL}. Some DC current does manage to cross the electrode-electrolyte interface and this leakage experiences a charge transfer resistance, R_{CT}. The expression for R_{CT} is derived from the Butler-Volmer equation;

$$R_{CT} = \frac{RT}{nF}\frac{1}{i_0} \quad (1)$$

Where R is the gas constant, T is the temperature (K), F is the Faraday constant, n is the number of electrons involved in the electrode reactions and i_0 is the exchange current density.

The charge transfer resistance is considered to be in series with the solution resistance R_s and in parallel with the double layer capacitance. Commercial software packages are available (e.g. Gamry, Scribner, and Solartron) providing equivalent circuits models such as Fig. 2 to model the experimental data. The software uses a constant phase element (CPE) instead of a pure capacitance to fit the experimental data. This is because biological responses produce a depressed semi-circular arc, where the intercept angle at high frequencies is not 90° as expected for a pure capacitance.

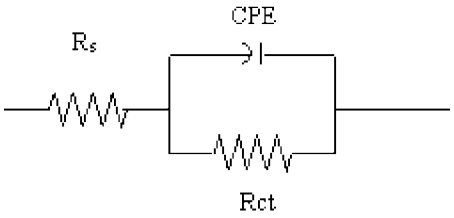

Fig.2. Equivalent circuit model, showing the bulk resistance R_S, the charge transfer resistance R_{CT} and a constant phase element CPE.

The use of CPE for biological systems has produced much better fits with experimental data than a pure capacitance as the electrode/solution interface exhibits non-capacitive properties.[7] The impedance of a CPE is defined in equation 2 where K is a measure of the magnitude of Z_{CPE} and has units of $\Omega\ s^{-\alpha}$, j is the imaginary unit ($j = \sqrt{-1}$), ω is the angular frequency ($\omega = 2\pi f$) and α is a constant which has a value between 0 and 1;

$$Z_{CPE} = K(j\omega)^{-\alpha} \quad (2)$$

Any complex plot where $\alpha<1$ is more accurately modelled by a CPE. There is no possibility of estimating the double layer capacitance C_{DL} from K values.[8] It is important to remember that the CPE exhibits no capacitive behaviour and the assumption that the double layer capacitance C_{DL} = K is incorrect and results in inaccuracies in all other modelled parameters.[7,8,9] Values of K in section III of this paper are presented as a function of the magnitude of Z_{CPE} at the interface.

III. RESULTS

A. EIS of Biofilm Staphylococcus epidermidis RP62A

Staphylococcus epidermidis RP62A adhesion to the gold electrode surface was visually confirmed following impedance measurements by staining the biofilm with crystal

violet. The electrode was washed in sterile de-ionized water and then suspended in 5% crystal violet in methanol. This procedure stains the biofilm cells deep purple. Following staining and prolonged rinsing the biofilm did not delaminate from the gold surface.

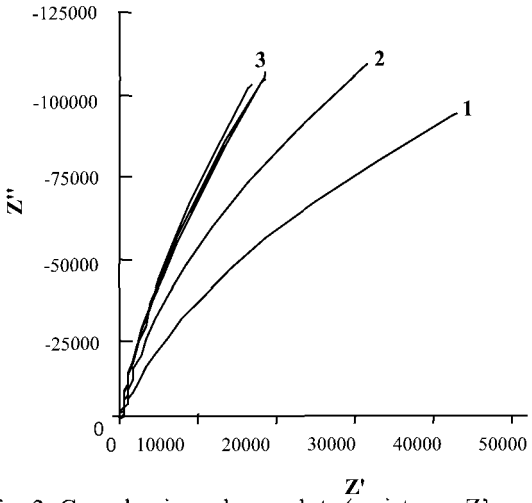

Fig. 3. Complex impedance plots (resistance Z' vs. reactance Z'') for sterile media (1), inoculation (2) and during biofilm growth (3).

Fig. 3 shows plots for blank media, inoculation point (t = 0 h) and during microbial biofilm formation. The shape of the data plots are different for pre and post inoculation. The major differences are observed between the blank and culture plots at the lower frequency end of the spectrum, where charge transfer resistance (R_{CT}) dominates. Figures 4, 5 and 6 show the modelled data for all complex plots for the 24 hour measurement period. The bulk resistance (R_s) of the media decreased over the 24 hour measurement period as shown in Fig. 4. At t = 0 h R_s = 264 Ω and at t = 24 h R_s = 170 Ω, a decrease of 94 Ω. This is explained by Cady's[2] theory that bacterial metabolism causes the breakdown of large organic molecules in the media into smaller more conducive ions which gives an increase in media conductivity over time.

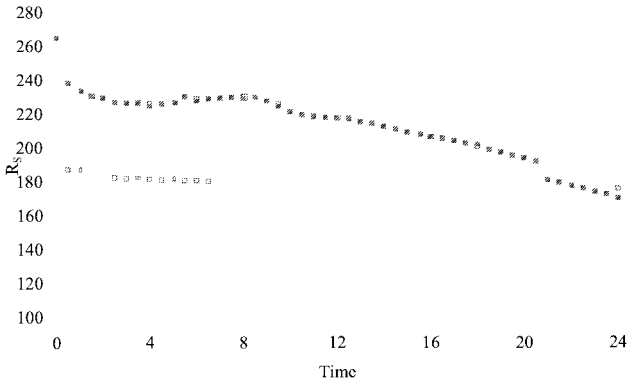

Fig.4. R_s (Ω) vs. Time (h) for sterile BHI media (□) and biofilm formation (■) as modelled by the Zview Software.

The decrease in bulk resistance was not as marked in the blank media, however R_s did show a decrease with time, at t=0 R_s=187Ω and at t=24 R_s=175 Ω, a decrease of 12 Ω. Values for K did not change markedly over time and remained relatively constant at 0.15 μΩ $s^{-\alpha}$ for the inoculated solution (Fig. 5). Values of K for the blank media were higher (0.2 μΩ $s^{-\alpha}$) compared to that of the inoculated media (0.15 μΩ $s^{-\alpha}$) at all time points, which shows that the presence of the bacteria decreased the impedance of the CPE at the interface.

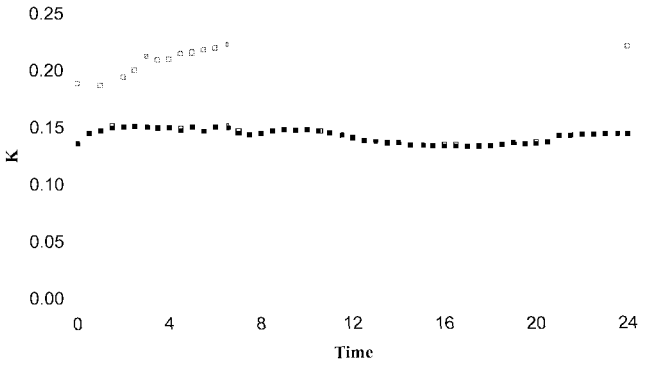

Fig. 5. K (Ω $s^{-\alpha}$) vs. Time (h) for sterile BHI media (□) and biofilm formation (■) as modelled by the Zview Software.

R_{CT} was observed to increase dramatically in the first few hours of biofilm formation (Fig. 6). A magnitude of 0.6 MΩ was recorded at inoculation which doubled after 2 h to 1.2 MΩ and continued to increase for the duration of the experiment. There was a small initial decrease in R_{CT} for the blank media in the first 30 min and thereafter the value remained constant at ca. 0.1 MΩ for the duration of the experiment. It is important to note that the complex impedance plots for this set of experiments (Fig. 3) are not complete semi-circular arcs and therefore values produced by the commercial software for R_{CT} are an approximation and subject to error.

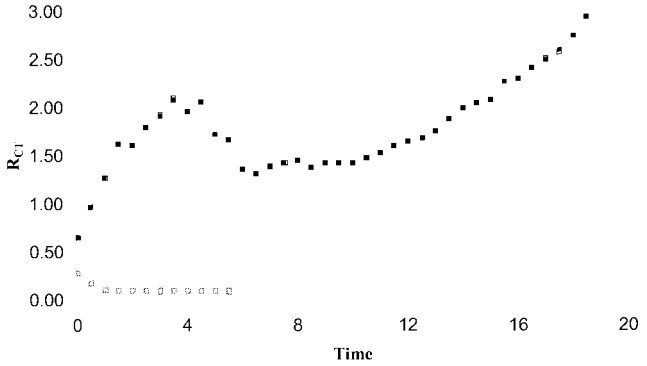

Fig. 6. R_{CT} (MΩ) vs. Time (h) for sterile BHI media (□) and biofilm formation (■) as modelled by the Zview Software.

Comparing the data for R_{CT} during biofilm growth with R_{CT}

values for blank media there is a marked difference in their pattern and magnitude. Further investigation at lower frequencies is required to determine if a change in R_{CT} could be used to detect initial biofilm adhesion and recruitment.

IV. DISCUSSION AND CONCLUSIONS

Microbial biofilms are defined as matrix-enclosed bacterial population adhered to a surface or each other. An organic matrix protects these sessile organisms.[10,11,12,13] Biofilms are characterized in part by the production of a network of highly hydrated extracellular polysaccharides (EPS). The EPS matrix has a number of functions, one of which includes facilitating the initial attachment of the bacteria to the surface. The EPS matrix has been reported to change the surface charge and free energy on the surface to be colonized and may account for changes observed in R_{CT}.[10] Changes in R_{CT} could be explained by microbial adhesion where adhesion to the surface is usually characterized by two phases[12]: (i) The reversible phase which occurs when the bacteria are very near to the surface but not in direct contact and includes electrostatic, hydrophobic interactions and Van der waals forces, (ii) the irreversible phase where binding to the surface is complete and includes dipole-dipole interaction and covalent bonding. It is possible that changes in R_{CT} in the first few hours of microbial growth (Fig. 6) relate to the reversible stage of the biofilm formation with the continuing increase in R_{CT} relating to the second phase of biofilm formation. An increase in R_{CT} was not observed in the blank media. However, partial arcs in complex plots do not allow for a good approximation of R_{CT}, and future work should include an investigation of lower frequencies (<10 Hz). Changes in the double layer are often related to biofouling and biocorrosion, of metal electrodes.[13] In this set of experiments the gold surface of the electrode did not become corroded. Within the field of biocorrosion biofilm formation is monitored sometimes over a period of thirty days and in future experiments impedance monitoring will take place over longer periods of time. The observed decrease in the magnitude of R_s over time follows Cady's theory where the breakdown of metabolites in the media due to bacterial respiration and growth causes an increase in the net conductance of the media and consequently a decrease in R_s.

Future sensor design should take into account that the biofilm does not adhere well to the substrate, preferring the gold electrodes, this biological parameter may be exploited to improve the sensitivity of the impedance technique. However, one must also be careful not to promote biofilm recruitment on implantable devices.

The electrode-medium interface warrants further investigation to determine the most effective parameter for monitoring biofilm formation and bacterial growth. This study confirms that electrical impedance spectroscopy is a possible method for the in-situ or possible *in vivo* monitoring of biofilm recruitment and/or bacterial growth.

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