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Development of a conducting polymer cell impedance sensor

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ABSTRACT

Research in to label free methods for biological analysis have brought interesting developments. Cell impedance spectroscopy has been one of the promising outcomes. Here we show the development of an 8-well impedance measurement setup and studied the use of conducting polymers as electrode material in cell impedance spectroscopy. We have developed devices using PEDOT:PSS electrodes and shown its advantages (lower impedance and faster to reach electrochemical equilibrium) over conventional materials, such as gold. It is observed through electrochemical analysis that the lower interfacial impedance is due to the low charge transfer resistance of PEDOT:PSS. MDCK cell proliferation experiments were performed using both types of electrode materials to provide a comparative result. We applied electrical modeling methods to understand the cell–substrate interactions and shown its applications in cell impedance spectroscopy. This study presents the development and advantages of cell impedance spectroscopy using conducting polymer electrodes.

1. Introduction

The need for label free and real time analysis in cell biology is constantly increasing. The nature of cell biological research requires parallel experiments in large numbers that yield cumulative statistical results. Research to meet these demands has led to many technological advances in fields such as lab-on-chip devices and instrumentation that process large work intensive experiments in a factory line style. Most lab-on-chip devices still require cells to be labeled before analysis [1]. Novel ways of detection along with parallel processing are required which do not interfere with the cells in culture. Cell impedance spectroscopy (CIS), a method pioneered by Giaever and Keese [2], provides such a possibility. CIS has been an evolving label-free tool for over two decades. It allows cells to be monitored in vitro and data can be collected for multiple experiments in parallel. Early research showed that the method could not only be applied for cell proliferation and motility measurements, but also be used to assess morphological characteristics (confluence of the cells) and behavioral aspects (such as metastasis) of the cells [3,4]. The applications and research for CIS have been growing rapidly including in field toxicological studies [5], drug testing [6], measuring mesenchymal stem cell differentiation [7–9], the measurement of cell substrate separation [10] and the monitoring of relaxation and contractility of muscle cells [11]. Electrodes for CIS have predominantly been based on noble metals. Conducting polymers have been researched thoroughly for antistatic coatings, electrodes (electrochemical capacitors [12], electrochromic devices, ion sensors [13]) and have been instrumental in new forms of biosensors [14].

Conducting polymers were first reported in the mid 20th century [15–17] but it was the discovery by Shirakawa et al. in 1977 that is considered the highlight for their discovery of polyacetylene [18] and earned him, along with Heegar and MacDiarmid, the Nobel Prize in chemistry in 2000. The application of conducting polymers in biological research are numerous and they are being extensively used for tissue engineering, neural probes, biosensors, drug delivery and actuators [19]. Commercially available conducting polymers, such as poly(3,4-ethylenedioxythiophene) (PEDOT), are commonly used as conductive coatings and intermediate layers in organic electronics [20]. PEDOT has the advantage of being transparent and biocompatible [21] while being considered the most stable (in the atmosphere and oxidizing environments) conducting polymer currently available [22–24]. It allows for simple and flexible methods for patterning, and also has the ability to be blended with other polymer compounds to create novel materials with unique properties [25,26].

PEDOT has been proven to have good electrochemical stability in phosphate buffer solutions, even when polarized [27,28]. It has been studied as a neural electrode coating and is considered one of the promising new materials with higher charge injection limits

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and better signal to noise ratio in measurements of neural activity [29–31]. PEDOT electrodes tend to delaminate over a period of a few weeks when in a saline solution [28], however Reza et al. found PEDOT nano tube based coatings to have improved adhesion of the coating to the substrate and the lower impedance provides better neural signal recordings [32]. They also found that the material improved the neural attachment and neurite outgrowth [33] and by coating a electrospun biodegradable polymer (loaded with drugs) with PEDOT, they were able to use electrical stimulations to release these drugs [34].

Most CIS setups work at frequencies where the higher impedance of nobel metal electrodes is of little concern. However lower frequencies can contain information useful for curve fit analysis and possibly distinguish the cell type. Not only can PEDOT as an electrode material for CIS provide a lower interfacial impedance but due to its transparency cells are accessible to high resolution microscopy, which makes this a uniquely suitable material for this application. This paper presents development of a cell impedance measurement device that utilizes PEDOT:PSS (solution processable PEDOT with poly(styrenesulfonate) (PSS) as doping agent) as the electrode material. The devices were fabricated using photolithographic methods. A complete impedance measurement setup was implemented in Labview which provides more flexibility in the device design and data analysis. A multiplexer was also designed and implemented for a multi well device that allows to measure eight experiments in parallel. As the design has the same electrode connection lengths, ensuring, that results for all the wells are not impacted by variations in inductance and series resistance of the material. The design shows the possibilities of creating simple and cost effective polymer based cell impedance measurement electrode devices.

Impedance measurements with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) saline solution showed a lower interfacial impedance of PEDOT:PSS electrodes in comparison to similar sized and shaped Au electrodes, highlighting the electrical advantage of PEDOT:PSS as an electrode material. Through electrochemical impedance analysis and curve fitting of the results to Randle's theoretical model [35] of an electrode–electrolyte interface, we determined that the lower charge transfer resistance of PEDOT:PSS as compared to Au is the reason for the reduced interfacial impedance. Experiments with the epithelial Madin-Darby Canine Kidney (MDCK) cell line showed that the PEDOT:PSS electrodes were fully capable of being used for CIS. Due to the fast charge transport capabilities of PEDOT:PSS, the electrodes quickly reach their equilibrium state. Curve fitting of the experimental data to typical electrical models of biological cells in series with the electrode equivalent circuit, show the versatility of CIS as a method to observe not only cell growth but also the cell–substrate interaction.

2. Materials and methods

Commercially available poly (3,4 ethylenedioxythiophene) poly(styrenesulfonate) (Orgacon S305 plus, AGFA) was used. It is a 0.54% by weight aqueous solution of PEDOT:PSS. The S305 plus includes binders to help adhesion to substrates and stabilizers for improved environmental stability. SU8-3005 (Microchem, Newton, MA, USA) and Microposit S-1818 (Shipley, Coventry, UK) photo resists were used for fabrication. EC solvent (Ethyl Lactate, Microposit, Shipley, Coventry, UK) was used to develop SU-8, and MicroDev (Microposit, Shipley, Coventry, UK) was used for S-1818 development after a 1:1 dilution with RO water. The glass substrates used were 50 mm × 75 mm microscope slides (Corning, Amsterdam, The Netherlands). Acetone, methanol and iso-propanol (Sigma Aldrich, Dorset, UK) were used for cleaning the substrates and devices at various steps. Wells were created using μ-slide 8-well culture dishes (Ibidi, Munich, Germany).

MDCK II cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with Medium 100, fetal bovine serum (FBS), sodium pyruvate and antibiotic all acquired from GIBCO, Invitrogen. Prior to cell seeding, the device wells were sterilized with 70% ethanol and rinsed with HEPES saline solution before being coated with poly-L-lysine (PLL Mol. Wt. 150,000–300,000, cell culture tested, Sigma, Poole, UK). Incubation during the cell culture and measurements was done at 37 °C in a 5% CO_2 environment. In certain experiments cell culture inserts (Ibidi, Munich, Germany) were used to seed cells in a specific area in contrast to a full coverage of the culture well areas.

2.1. Instrumentation and software

Impedance measurements for the electrode comparison were taken using a QuadTech 1420 LCR meter (from 20 Hz to 1 MHz; Quadtech, Marlborough, MA, USA). Cell impedance measurements were done using an Agilent 4294A impedance analyzer (Agilent, Berkshire, UK). The impedance measurement equipment was connected to the wells through a multiplexer. The multiplexer was designed with four 1:16 channel switches (two ADG726, Analog Devices). By keeping the multiplexers separate for each of the four current and voltage probe lines, the configuration further negates any effects due to the multiplexer. The ADG726 package has an "ON" resistance of only 4 Ω and does not mitigate the accuracy of the system. A NI-DAQ 6211 (National Instruments, Berkshire, UK) provided the digital signals to the multiplexers and a single power supply was used to provide positive and negative voltage to linear voltage regulators, which feed the rest of the circuitry. The multiplexer board holds the device and both sit inside an incubator. The setup is controlled using software designed on Labview (National Instruments, Berkshire, UK). The amplitude of the signals used for the electrochemical comparison was 20 mV, which is low enough to work in the linear region of the current and over-potential relationship [36,37]. The amplitude for CIS measurements was kept higher (80 mV) which was set after it was observed to be free of any noise when measured using both types of electrodes. It is small enough to be free of harmonic distortion and neither caused any adverse effect to the cell culture.

2.2. Device fabrication

A single device had eight wells, arranged in four columns and two rows. Each well had a pair of electrodes: a working electrode and a reference electrode. The design of the electrodes was similar to those available from Applied Biophysics ECIS systems with a large reference electrode and a circular working electrode whose dimensions and geometry are defined by the windows created in the insulation layer. The devices (Fig. 1) were fabricated using photolithography on a glass substrate. The glass substrates were cleaned using acetone, methanol and propane-2-ol, 5 min in an ultrasonic bath, followed by oxygen plasma cleaning (GaLa Instrumente, Bad Schwalbach, Germany) for 3 min at 160 W. An alignment layer was deposited on the substrate via a lift off process involving S-1818 lithography and aluminum deposition by evaporation using a Plassys metal evaporator (Plassys, Marolles En Hurepoix, France). Fig. 1 shows the fabrication process for the PEDOT:PSS electrodes and the SU-8 insulation layer. PEDOT:PSS was spin coated at 1000 RPM for 30 s onto the substrate. This was done twice to achieve approximately 100 nm thickness. The sample was baked at 95 °C for 1 min between the two layers and for 5 min after the final coating. S-1818 photo resist was then spin coated and baked at 95 °C for 5 min. After lithography and development (90 s) of S-1818, PEDOT:PSS was exposed to 5% sodium