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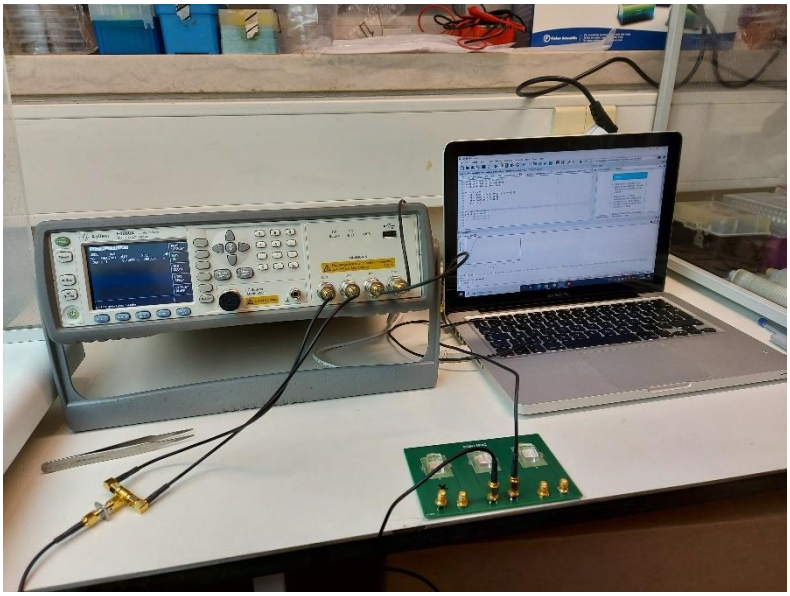

Impedance monitoring of cell cultures in real time in a biochip using integrated microelectrodes

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Abstract

The present work was focused on impedance measurements in cell cultures in microchips to better understand cell growth, cell proliferation and cell removal on a chip.

This was achieved using interdigitated microelectrodes in which cells were cultured and studied using impedance measurements.

The biochip was developed using microfabrication techniques and after which it was used for cancer cell seeding and culture with the aim of mimicking the conditions of cell growth in vivo. This was achieved and impedimetric measurements occurred.

To further understand the behaviour of the impedance of high concentration solutions and cell cultures, experiments using PBS, NaCl, E-Coli and IgG were conducted.

In this work it was possible to distinguish the impedance behaviour of solutions with low concentrations and higher concentration, such as the difference between DI Water and PBS.

It was also possible to grasp the impedimetric behaviour of suspensions of solid substances like E-Coli and IgG. However this is only possible in very low concentrations of solutions as the behaviour of the higher concentration solutions is the dominating factor in the impedance measurement.

It was possible to conduct the cell culture with the microfluidic device, however due to fact that the culture medium utilized for the cell culture has a high concentration of nutrients and other molecules it was not possible to detect the cells within the microchip as well as their growth, proliferation and removal.

As a result, the devices should be optimized for impedimetric measurements.

1. Introduction

Cell cultures and their properties have been widely studied in regard to standard cell culture techniques, like Petri dishes and microwells. However, in cell biochips, cells are cultured in microfluidic chambers. The integration of the cells in microsystems has the advantage of allowing a very high level of geometrical, fluidic, and chemical control and thus a high level of control of the cellular microenvironment. This could mean that the process could be faster, less complicated, and cheaper.

Cancer research is a particularly important field and in high demand for drug screening and testing on a fast a big scale, as different patients respond to different drugs. As a result, Lab-on-chip could provide the means to achieve that by a being able to monitor cell growth, proliferation, and death in a real-time scenario. This means that different drugs and concentrations can be tested faster and cheaper whilst being able to provide the patient with the necessary means of treatment.

Impedance is the characteristic of an electrical circuit that resists the flow of charges and is used in engineering to relate with the alternating current (AC). The frequency response can be split into two parts: the amplitude response and the phase response. The former describes how the amplitudes of the harmonic oscillations at different frequencies are damped or amplified by the system. The latter, the phase response, describes the phase shift of the system and is important for the valuation of the duration of the vibration component though the system. They can be graphically depicted in a Bode plot, where the amplitude response is plotted on a log-log graph and the phase response is plotted on a log scale. The impedance spectrum can also be plotted in a Nyquist plot by its real and imaginary parts. Then, though Impedance Spectroscopy, the complex electrical resistance (impedance) of a system as a function of the frequency can be measure by exciting the system with a small amplitude sinusoid signal and varying the frequency. The Bode plot in then used to analyse the resistive, capacitive, and inductive behaviours of the system

For the monitorisation of cells in a microfluidic chip with electrodes, the cell culture needs to be supplied with salts and nutrients, forming an electrolyte solution that has a solution resistance. The double layer is formed between the electrodes and the surrounding electrolyte, which is the double layer capacitance and there is a charge transfer and diffusion layers. This is the easiest equivalent circuit referred to as Randles equivalent circuit for an electrode electrolyte contact.

Conventional culture methods are well established processes, but difficulties related to static conditions, not accurate representation of real cell environments, unwanted concentration gradients and cost can be overcome with microfluidics. The implementation of continuous flow can supply cells with media continuously through pumping systems, which prevents gradients in pH and concentrations. On microfluidic devices, smaller volumes are required, and sensors can be included into the systems, reducing production and reagents costs and analysis time, as well as being user-friendly, sensitive, and providing reproducible results and conditions.

Like all devices, there are certain advantages of using microfluidic chips for cell cultures such as the responsiveness of the devices, which are faster than their normal sized counterparts, the lower consumption of reagents and other substances required, the ability to monitor several different conditions of study as well as the mimicking of *in vivo* cellular microenvironments that is fundamental to study and understand the cell cultures, such as the reaction to different nutrients, vascularization and interactions between cells.

On the other hand, this strategy can also have its disadvantages, one of them being the fact that laminar flow, a rather slow process, is the limitation factor in diffusion of the nutrients to the cells. This results in a concentration gradient within the cell chip that is undesirable for the necessary growth conditions of the cells. Moreover, while the microfluidic devices themselves might not be expensive to fabricate the production materials and equipment's as well as the specialized personnel increase the overall cost of these devices.

2. Materials and Methods

In order to perform these experiments it was necessary to fabricate the microchip. The steps for the microfabrication are explained in this section and the preparation of the microchips was conducted in a class 100 cleanroom (100 000 particles over 1 μm per m^3).

The interdigitated electrodes were produced with a thin layer of Indium Titanium Oxide (ITO) deposited on a glass substrate, covered with a photoresist layer that was structured with exposure and development and the metal layer was structured using the resist as an etching mask. The preparation of the substrate includes several steps such as its characterisation (i.e. roughness), surface treatment (i.e. adhesive properties) and cleaning to remove any and all contamination, with the latter being an ever-present step regardless of the use given to the substrate. To imprint the wanted structure onto the ITO film the technique used was photolithography and the design was made using AutoCad software. The dimensions of the interdigitated electrodes and the design of the structures had previously been done and optimized. After the photolithography the result is a substrate with six interdigitated electrodes in a 7cmx7cm glass substrate, with one of the electrodes being designed shorted to serve as reference for measuring the resistance. This implies that dicing is required.

The fabrication of the microfluidic structure involves several steps such as the hard mask fabrication, the master mould fabrication and the production of the (Polydimethylsiloxane) PDMS structures as well as the sealing of the electrodes.
The components of the hard mask of this structure are PDMS, which is a transparent substrate, and the previously structured ITO layer. The hard mask was fabricated using photolithography and etching. The master mould for this structure was fabricated using photolithography. The final microfabrication step is the PDMS structure fabrication. It is important that the microchamber structure is even in order to allow optimal cell growth and flow within the channels After the PDMS structure is made, it is bonded to a glass structure

In order to be able to perform the impedance measurements the integrated microchip needs to be electrically connected to a measuring platform, In this case it was a printed circuit board (PCB) that was designed for the fabricated electrodes and taking into consideration the necessity of being able to fit into a microscope for scanning the whole microchip and to connect to the measuring instrument through SMA cables with SMA connector pins that were later added to the PCB. In most cases copper was added between the metal pins and the PCB to enhance the connection. The microelectrodes were wire bonded to the PCB and a multimeter was used to

verify the connection the gold pads of the PBC that is connected to the electrodes and the connectors.

The measurement of impedance of cells was conducted after cells were cultured in the fabricated microchips, in which they grew and multiplied and then injected with phages. The cells used were from colorectal carcinoma (CRC cells) from the HCT 116 cell line and are immortal and adherent. Cryopreservation is used to maintain the cell lines.

The measurements of impedance are made with and LCR meter and using a python script previously developed. Before any measurement could take place, a correction was performed with a metal object and the LCR could not be turned off until the last measurement was made several days later to maintain the measurements relative to the same correction. The impedance measurements are run at 201 frequency points increasing from 20 Hz to $2x10^6$ Hz. The measurement of culture medium and DI Water after the removal of the cells serve as a means to monitor the behaviour the these solutions and the end of the experiment by comparing these measurements with the ones made before inserting the cells, as well as being indicators if the washing of the microchip, particularly the measurement of the DI Water at the begging and the end.

3. Results

Before the experiments with cell could happen, it was necessary to understand the behaviour that more simple solutions have in regard to impedance as the main aspects of culturing HCT 116 on the microchip was already known from previous works. As a result, solutions with increasing complexity and concentration were studied.

The first solutions to be measured were DI Water as well as PBS and NaCl with increasing concentrations. It was possible to observe that the resistance of the solution decreases with the increase of concentration whereas in the case of the resistance of the charge transfer the opposite happens, as the value increases as the concentration does so as well. This happens because with higher concentration of the same solution we have more free charges and therefore more resistance due to the increase of the number of molecule complexes that are formed. On the other hand, the more charges mean that the solution is less resistant.

The next step was to measure the effects of solid substances in solutions of which the behaviour of impedance was already known, DI Water, and in the culture medium that would be used for the cell cultures in the experiments with cells. For this to happen, solutions of bacterial cells, E-Coli, and proteins, IgG, were prepared with different concentrations. A clear distinction of the different concentrations of E-Coli and IgG can be had in the lower concentrations in DI Water. However with solutions of higher concentration, there is little difference in behaviour and values of resistance. This is even more evident in solutions of culture medium and E-Coli and IgG. This happens because the solution of culture medium itself is more prevalent than the cells in the solution, and as a result is the substance of which the impedance is being measured

The experiments with cells occurred in a duration of roughly 48h and several impedance measurements were made before, during and after cell culture. Five runs of experiments were conducted to varying degrees of success and slight alterations to the protocol. However all were conclusive in the fact that cell growth monitoring and detection are not detectable using these microelectrodes and culture conditions. There is very little difference between any of the measurements during the cell culture, regardless of the time, and the behaviour of the culture medium.

4. Conclusion

With this work, the focus was to be able to detect cell growth and proliferation and detection with impedance spectroscopy. For this purpose, an interdigitated ITO microelectrode was used for the cell culture and subsequent impedance measurements. In order to achieve this, the first step was to study and monitor the impedance behaviour of less complex and concentrated solutions.

Cell culture inside the microchip and cell removal were possible. However, the microelectrodes that were fabricated and had been used previously and achieved good results for cell culture did not have the initial aim of impedance spectroscopy experiments and therefore the microfluidic device was not the most optimised for this end.

Factors that may have contributed to this situation and that can be further studied are, for example the size of the chamber, the material of the electrode, the fact that the inlets and outlets of the microchamber and the posts of the microfluidic device interfere with the electrodes and not creating a gelling of the 3D culture as to have more electrical stability.

When the cell culture experiments are successful in identifying the several stages of cell proliferation and growth as well as removal, only certain frequencies should be monitored as to be able to administer different types of drugs and successfully identify each and every one by their impedance measurements thus creating and effective drug screening tool.

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