Electrical Characterization of Biological Cells on Porous Substrate using COMSOL Multiphysics

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Abstract: In this paper, the gross electrical characterization of biological cells on porous substrate using COMSOL Multiphysics is analyzed. Dynamic electrical characterization during cell growth is required as non invasive and label free technique to understand the growth kinetics of cells. It is observed from COMSOL simulation that the percentage change in current density is greater in porous substrate than that of flat substrate. Further impedance variation of normal keratinocytes (HaCat) has been carried out in real time with our proposed porous silicon substrate to validate the simulation results.

Keywords: ECIS, Porous silicon, Electrical characterization

1. Introduction

The dynamic analysis during cell growth is required to understand the growth kinetics of cells. The commercially available platform called electric cell substrate impedance sensing (ECIS) for this purpose was first reported by Giaever and Keese [1]. Electrical monitoring of the temporal responses of biological cells cultured in different conditions under the application of variable frequency, low intensity sinusoidal electric fields has been extensively employed as a label free quantitative method to study various cell biological processes like cell attachment, spreading, cell growth, proliferation, cell apoptosis and cell micromotion [2-7]. ECIS platform has been widely used to monitor different cellular activities like cell adhesion, spreading, proliferation of cell in real time [8]. The cytotoxic effect on attachment and spreading of fibroblastic V79 cells cultured on small gold electrodes precoated with fibronectin is detected as electrical resistance changes [9-10]. A real time and continuous technique based on electric cell-substrate impedance sensing (ECIS) has been developed for measuring the cellular cytotoxicity [11]. The novel electric cell substrate impedance sensing technique is used to monitor cell adhesion/spreading, barrier function and wound healing. Primary bronchial epithelium is compared with airway epithelial cell lines 16HBE14o-, BEAS-2B, NCI-H292 and A549 [12]. ECIS platform has already been used to monitor different cellular activities. But the different stages of growth cannot be distinguished apparently. Extensive models have to be used to extract the cell parameters at different stages. Axonal outgrowth of Dorsal Root Gangiala (DRG) on smooth and porous silicon surfaces has been studied. The outgrowth depends on the size of the pores [13]. The adherence and subsequent viability of rat neuronal B50 cells has been carried out on the nanostructured porous silicon (PS). This study indicates that PS offer advantages over bulk Si surfaces for neuron cell adherence and viability [14]. But dynamic electrical characterizations of biological cells have not been carried out with porous substrate so far.

So far, different flat substrates are used for dynamic electrical characterization of biological cells. In this paper, we report the gross electrical characterization of biological cells on novel porous substrate using COMSOL Multiphysics.

2. Design and Simulation using COMSOL Multiphysics

A finite element method based commercial software package, COMSOL multiphysics, is used to produce a model and study the total current density variations with different cell numbers for rectangular as well as circular electrodes on flat and porous substrate. The software provides an integrated geometry and graphical user interface for preparing the model, a computational solver for performing the simulation, and an interactive visualization program. In this study, the simulations are performed using the three-dimensional Conductive Media DC module under the electrostatic model of COMSOL Multiphysic. The 3D model has been constructed in the

drawing mode of COMSOL Multiphysics. The top view of cells on top of the pores with rectangular electrode and circular electrode are shown in Figure 1 and Figure 2 respectively. The size of cubic pore is 2×2×2 µm each. The radius and height of each cylindrical cell is 2 µm. During simulation, subdomain settings and boundary settings under physics menu are set.

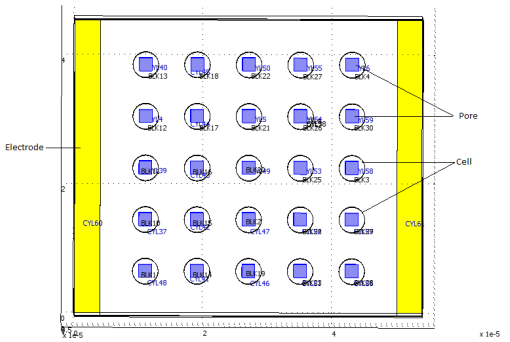

Figure 1. Top view of cells on top of the pores with rectangular electrode

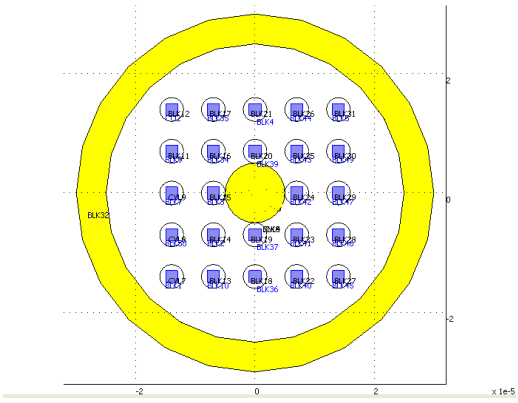

Figure 2. Top view of cells on top of the pores with circular electrode

The boundary conditions are set as electric potential of 0.1 V on one electrode and ground on another electrode. In subdomain settings, conductivity is given as input variable. The conductivity of solution and cell are set as 10 S/m and 0.01 S/m respectively. Two different types of electrodes are taken here. One is parallel electrode, another is circular electrode. Three distinct simulations have been performed for each electrode: (i) cells on flat substrate, (ii) cells on top of the pores and (iii) cells outside the pores. The percentage change in total current density with number of cells for rectangular electrode and circular electrode is shown in Figure 3 and Figure 4 respectively. It is observed from these two figures that the percentage change in current density is greater in porous substrate than that of flat substrate. This can be attributed to fact that the current lines passes through the pores by following the lower resistive path. So in presence of cell the impedance variation is larger in porous substrate than the flat substrate.

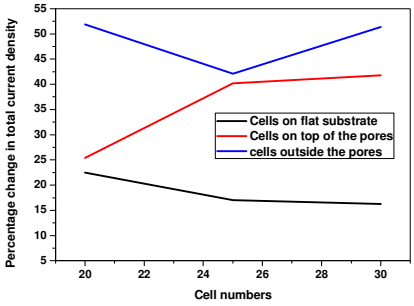

Figure 3. Percentage change in total current density for rectangular electrode

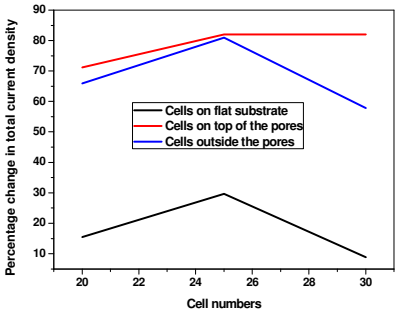

Figure 4. Percentage change in total current density for circular electrode

3. Materials and Methods

3.1 Fabrication

Mirror polished silicon wafers of p-type having resistivity 10-20 Ωcm has been etched electrochemically with a mixture of hydrofluoric acid (HF) and dimethyl sulfoxide (DMSO). A platinum plate is used as cathode. The samples are then anodized by a constant current power

supply (with current density 2 mA/cm^2) for one hour. Thus porous platform has been formed. After formation of porous silicon, rectangular metal electrodes are made on top of this substrate. First, Ag-paste is used to form the electrode with the help of screen printing. Then the sample is treated at 750°C for 1 minute. After that, metal mask is used to cover the entire portion of the substrate except the electrode area. Then gold evaporation has been done by the vacuum coating unit Hindhivac B600 to form a thin layer of gold on the electrode. Then it has been baked for 10 minutes at 110 °C. The wires are connected from the metal pads by soldering. Finally a polydimethylsiloxane (PDMS) well is fabricated by soft lithography technique and composed over the porous area for containing the cell suspension. The process flow of the entire fabrication process is shown in the Figure 5. Figure 6 shows the photograph of fabricated device.

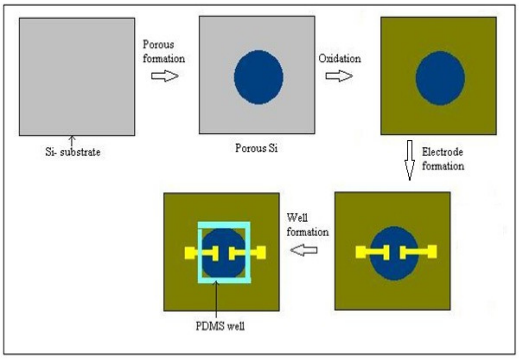

Figure 5. Process flow of device fabrication

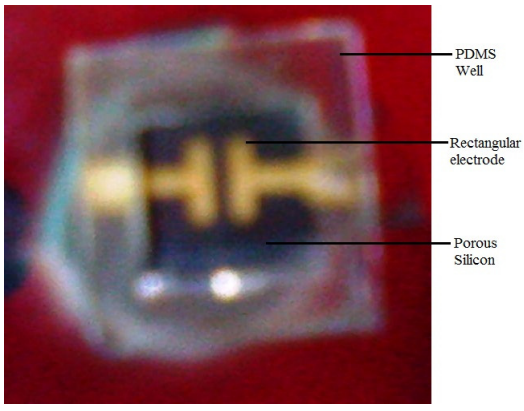

Figure 6. Photograph of the porous silicon device

3.1 Cell Preparation

HaCat cell has been seeded in a cell culture flask with DMEM-F12 media and incubated at 37°C in 5% CO_2 maintaining appropriate culture condition. After cell confluence, it has been trypsinized and centrifuged for 2 minutes at 2000 rpm. The media has been discarded and cell palette is resuspended in 2 ml of DMEM-F12 media. Then the solution of HaCat cell has been poured into PDMS well with concentration 2×10^5 cells/ml and the porous platform has been kept inside the incubator (Heal Force, HF-90). Then the readings of impedance have been taken with time at different frequencies for 16 hours. It shows that the impedance reading is changing with time for a particular frequency.

4. Results and Discussions

For measuring the impedance, DMEM-F12 media is poured into PDMS well onto the porous substrate with rectangular electrode using micropipette and after it has stabilized, the media is discarded. Then 200 µL of the cell solution is poured into the well and the electrical impedance reading is taken with the impedance analyzer (GW INSTEK LCR-821) in different time step at 4 KHz frequency for 16 h duration. As a frequency of 4 kHz is suitable for many applications like wound healing experiments [15] and very low frequency is not desirable for long term measurements [16], we take the electrical readings at this frequency. The impedance variation with time for blank medium and HaCat cell solution is plotted in Figure 7. It has been observed that the impedance of the blank medium is almost uniform with time but the impedance of the cell solution is increased gradually. This can be attributed to fact that the cell membrane is insulating in nature. During growth process, first, the cells are attached with protein coated substrate then it will grow and proliferate by increasing the cell numbers. It then covers the electrode area gradually with time by hindering the current flow [17]. So impedance of cell solution increases with time. It has also been observed that after 14 hours from starting of experiment the impedance value of cell solution saturates. This may be attributed to fact that after 14 hours of cell seeding it starts to be confluent. So there are no significant changes in impedance

value. After 16 hours from cell seeding the cell layer become totally confluent which is shown in Figure 8.

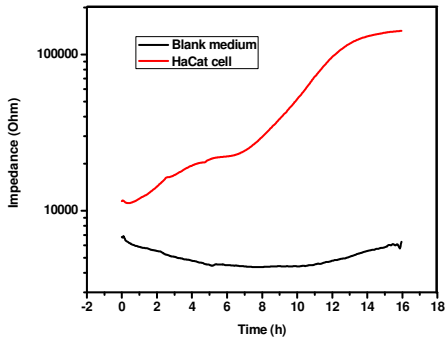

Figure 7. Impedance variation during cell growth with time at 4 KHz.

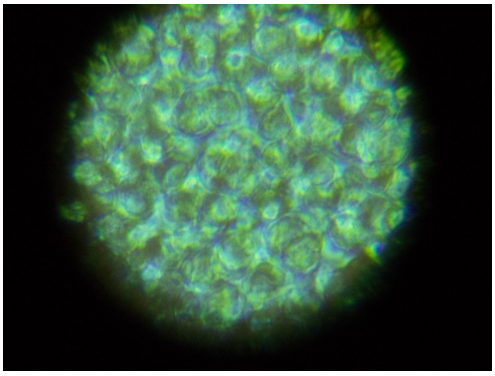

Figure 8. Optical microscopic view of confluent layer of keratinocytes (HaCat) cells after 16 hours from starting the experiment

5. Conclusions

This paper reports on the gross electrical characterization of biological cells on porous substrate using COMSOL Multiphysics. It is observed from COMSOL simulation that the percentage change in current density is greater in porous substrate than that of flat substrate. The novel porous silicon substrate has the potential to provide the information about the impedance variation with time during cell growth.

6. References

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