INVESTIGATION OF ENDOTHELIAL GROWTH USING A POLYCARBONATE BASED MICROFLUIDIC CHIP AS ARTIFICIAL BLOOD CAPILLARY VESSEL WITH INTEGRATED IMPEDANCE SENSORS FOR APPLICATION IN CANCER RESEARCH

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ABSTRACT

To extend the measuring capabilities of our two-layered microfluidic chip which can be used as artificial blood capillary vessel for *in vitro* examinations of the migration of cancer cells [1] we have structured gold electrodes on top of the separating membrane using optical lithography and chemical etching to perform impedance measurements. This allows the investigation of the growth and the barrier properties of the endothelial monolayer on top of the membrane which is used to mimic the blood vessel wall. First experiments show an accordance between microscopically detection of the cell growth and the electrical impedance measurements.

KEYWORDS: Micro Fluidic Chip, Membrane, Gold Electrode, Impedance Spectroscopy

INTRODUCTION

The process of haematogenous and lymphatic metastasis occurs, starting from the primary tumor, with the entry of tumor cells into the vasculature (intravasation). It follows the hematogenous or lymphatic spread of cancer cells, the endothelial adhesion, the transendothelial migration out of the vasculature (extravasation), and finally the formation of metastasis. For that reason, we developed a microfluidic two-channel system based on polycarbonate (PC) due to its excellent biocompatibility and optical transparency, consisting of two layers, an upper layer with two sets of three microstructured channels, simulating the blood vasculature, and the lower layer, which consists of two larger channels filled with artificial tissue (c.f. Figure 1) [1]. Both layers are separated by a porous membrane. To mimic the blood vessel wall, the upper surface of this membrane is seeded with a monolayer of human endothelial cells.

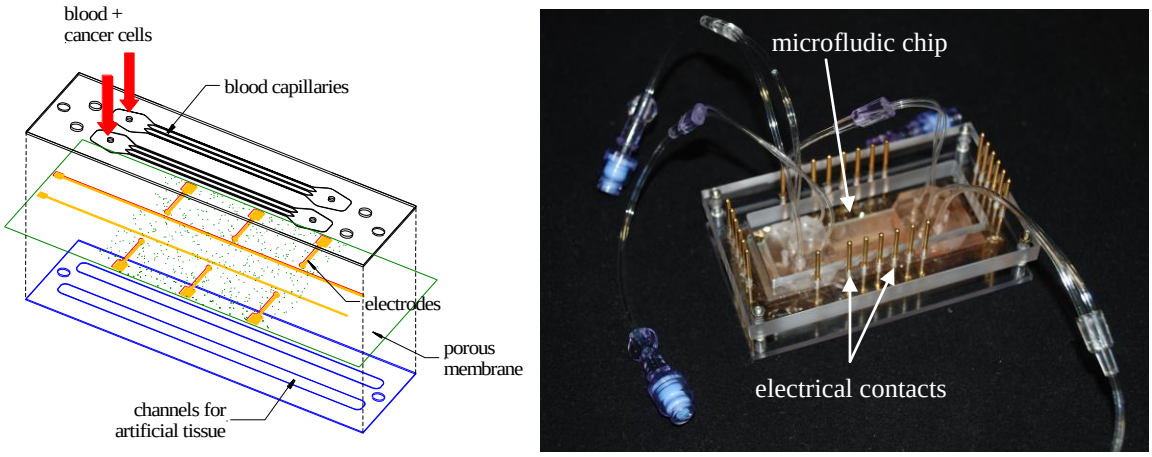

Figure 1: Schematic view of the artificial blood vessel system with integrated sensors (left). Micrographs of the microfluidic chip inclusive of the measuring device (right)

Up to now the growth of the endothelial cells was investigated by phase contrast or fluorescence microscopy. To improve the investigation of the growth and the properties of the endothelial cells gold electrodes were integrated on the membrane which allow impedance measurements of the cell layer [2-4].

THEORY

The impedance spectroscopy, especially the Electric Cell-substrate Impedance Sensing (ECIS) is an accurate method to examine the electrical properties of membranes or cell layers. The basic idea of the ECIS method is to cultivate adherent cells on planar gold film electrodes, which are located in a cell culture medium. By applying an AC voltage to the electrodes the impedance can be determined (c.f. Figure 2a). As soon as the endothelial cells adhere to the surface of the gold electrodes, the cell bodies act as insulating particles and impede the electrical current between the electrodes and through the surrounding medium. The process is identified by an increase of impedance. After a certain time when the cells have adhered completely, a cell-layer specific impedance value is constituted. The impedance is increased addition-

ally by the fact, that the endothelial or epithelial cells form cell-cell contacts by tight junctions. In general, the cell shape and the architecture of the cell type-specific cell-cell and cell-matrix contacts determine the final impedance value. As soon as the cells are stimulated by cancer cells or by addition of active agents, the cells shape change, which is displayed in change of the impedance.

EXPERIMENTAL

To perform the impedance measurements, gold electrodes have to be structured on top of the porous membrane using optical lithography and chemical etching. Finally an insulating layer has to be deposited on top of the whole and structured lithographically membrane to insulate the electrical pathways. Two different types of insulating material were tested: a standard photoresist AZ 4533 and polymethylmethacrylate (PMMA). For proof-of-concept measurements of the functionalized membrane we developed a special test chamber with lateral feed through for the electrodes to be connected externally. The reservoir above the membrane was filled with phosphate buffered saline (PBS) as electrolyte. The first measurements were performed with cell culture medium, gelatine and with cultivated endothelial cells, respectively. The experimental setup comprise either a Platinum wire in the cover plate of the test chamber as counter electrode or integrated counter electrodes on the membrane as shown in Figure 2b. The measurements were carried out in the high frequency range (10^2 – 10^5 Hz).

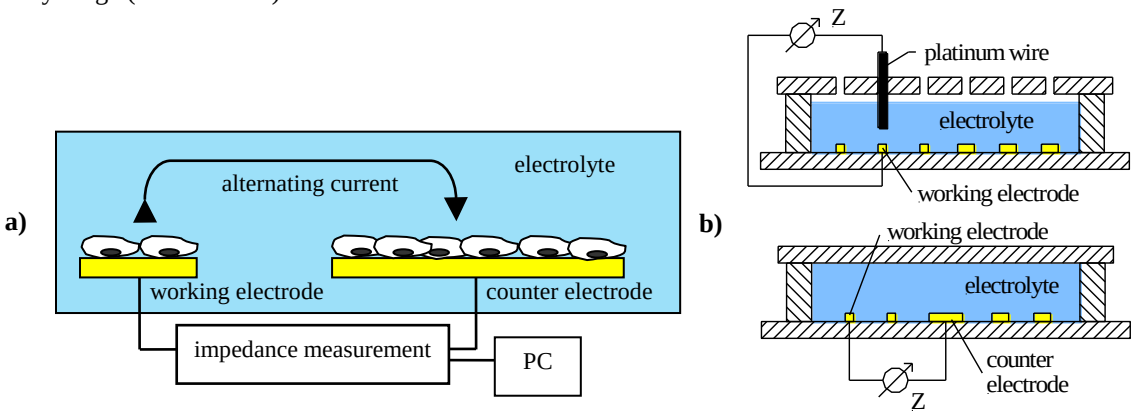

Figure 2: Schematic view of (a) the impedance measurement and (b) the test setup

For medical experiments, the test chamber was washed twice with distilled water. Then the test chamber was disinfected by means of UV light, because the photoresist coating dissolves with various types of alcohol. After the disinfection the chamber was filled with 2 ml cell culture medium. The medium remained for 12 hours in the test chamber at 37°C in a moisture rich environment. Afterwards the medium was aspirated and the chamber was coated with 2 ml preheated gelatine. The gelatine served as an artificial matrix supporting endothelial cell cultivation. Endothelial cells were seeded after 30 minute of with 1 million cells.

RESULTS AND DISCUSSION

Prior to the cell growth experiments, the test chamber was filled with gelatine and cell culture medium. All of these processes have been performed in an incubator under constant parameters (5% CO_2 and 37 °C). The measurements show that gelatine has a high impedance (c.f. Figure 3a). However, the cell culture medium showed a lower impedance (c.f. Figure 3b). As expected, large electrodes had a smaller impedance than small electrodes. We could also demonstrate that the parameters temperature and CO_2 saturation change the absolute value of the impedance causative for avoidable measuring inaccuracies.

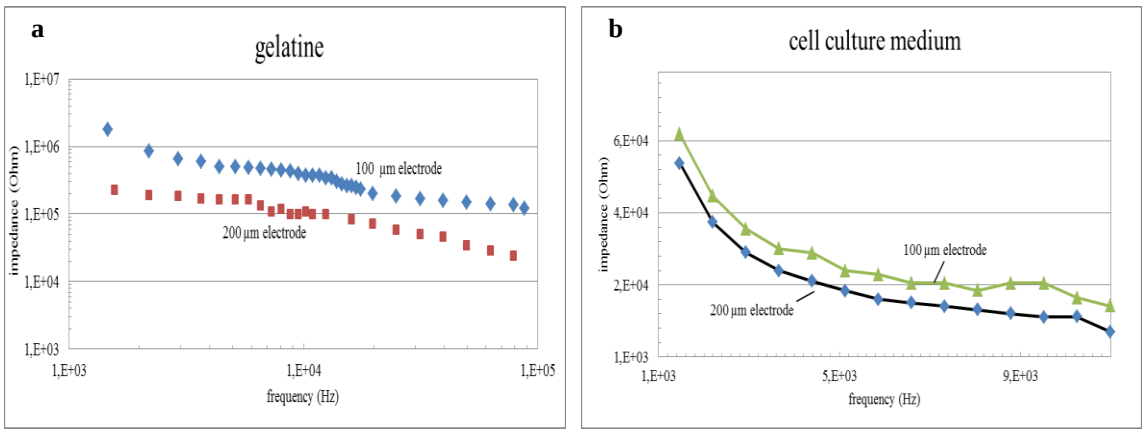

Figure 3: Impedance between the individual electrodes with (a) gelatine and (b) cell culture medium

Figure 4 compares the impedance spectrum of cultivated endothelial cells after 3.5 hours in the microfluidic test system (c.f. figure 4a and 5a). As expected, there is an increase of the impedance with the presence of cells. As the cells adhere and spread on the electrode surface, they impede the electrical pathway, thus increasing the impedance measurement. The measurements show that the impedance is increased after 24 hours of endothelial cell growth (c.f. figure 4b and 5b). Afterwards the test system was filled with 2 ml of EDTA-trypsin (cell-detaching agent), abolishing the cell-cell tight junctions to examine the influence on the impedance (c.f. Figure 4c and 5c). Finally the cell culture medium was removed and the cells died which results in an additional decrease of the impedance. The impedance measurements were accompanied by microscopical examinations of the endothelial growth. Figure 5 shows the microscopic recordings of cell growth. The successful cell growth is also an indicator for the biocompatibility of the selected photoresist.

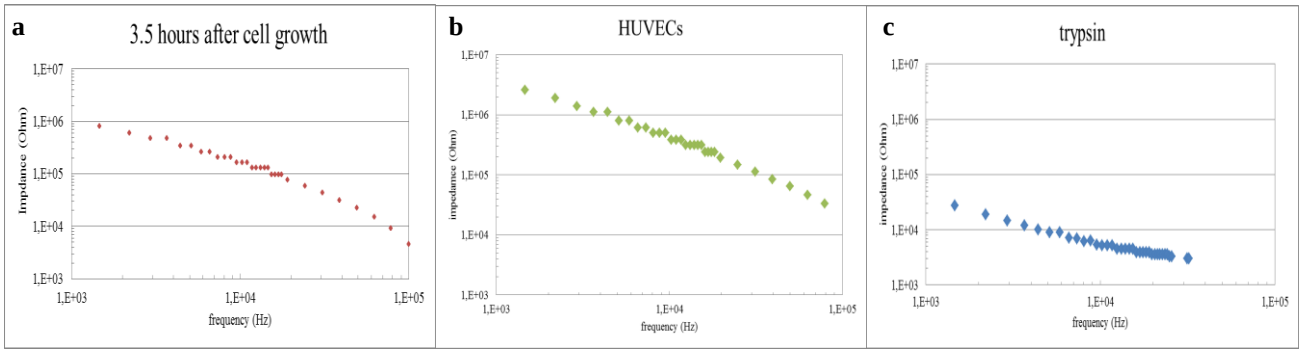

Figure 4: Impedance of cell growth (a) after 3.5h, (b) after 24h and (c) impedance with EDTA-trypsin

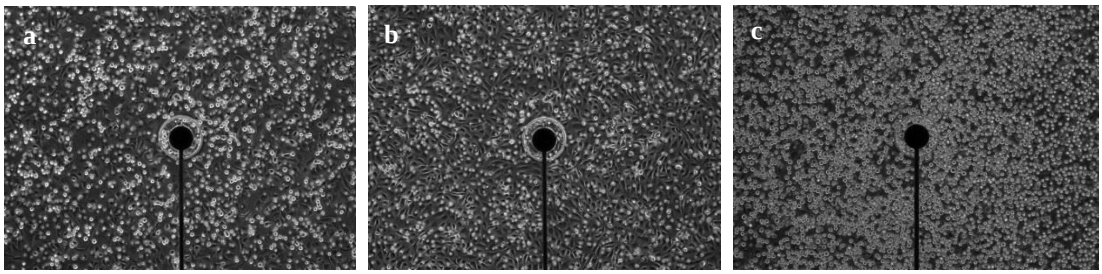

Figure 5: Photographs of an electrode after (a) 3.5h of cell growth and (b) 24 h of endothelial cell growth. (c) After filling with EDTA-trypsin endothelial cells have completely lost their contact to the surface and float in the medium.

CONCLUSION

We could show that ECIS measurements enable a detailed characterization of cellular monolayers and therefore an investigation of a ripening endothelial cell layer upon shear flow conditions..This developed integrated ECIS measurment system could be used as a real-time measurement technique next to optical observation methods. The next step is to verify the experimental results in the test chamber with the final two-layered microfluidic chip system. The aim is to characterize the impedance modifications of an intact endothelial cell layer by the interplay with stimulating agents and finally by floating and adhering cancer cells.

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