DEVELOPMENT OF A MICROFLUIDIC CHIP AS ARTIFICIAL BLOOD CAPILLARY VESSEL WITH INTEGRATED IMPEDANCE SENSORS FOR APPLICATIONS IN CANCER RESEARCH

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ABSTRACT

We have developed a microfluidic two-channel system based on polycarbonate (PC) which can be used as an artificial blood capillary vessel to investigate the transmigration of cancer cells under flow conditions and defined shear rate using live cell microscopy [1]. The system consists of an upper channel system mimicking the blood capillary and a larger lower channel simulating the surrounding tissue. Both channel systems are separated by a porous PC membrane which is coated with a monolayer of endothelial cells to mimic the blood vessel wall. The aim of this work is to integrate gold electrodes on top of the membrane which can be used as impedance sensors to detect the endothelial growth.

KEYWORDS: artificial blood vessel, impedance measurements, hot embossing, thermal bonding

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 from the vasculature (extravasation), and finally the formation of metastasis. Due to the tumoricidal action of the human immune system in the blood stream and the prevailing shear forces an early attachment and extravasation of tumor cells and the underlying tumor endothelial interaction is of particular importance. Conventional transmigration chambers for use under static conditions [2] or microscopic flow systems [3] allow studying the aspects of the extravasation only, without taking into account the influence of all environmental conditions within the microcirculation. For that reason, we developed a microfluidic two-channel system based on polycarbonate (PC), consisting of two layers, an upper layer with two sets of three micro structured channels with a width of 300 µm each and with heights in the range of 50 µm and 100 µm, simulating the blood vasculature, and the lower layer, which consists of two large channels that are filled with artificial tissue (cf. Figure 1). Both layers are separated by a porous membrane with a specific pore density distribution. To mimic the blood vessel wall the upper surface of this membrane is coated with a monolayer of human endothelial cells.

Polycarbonate was chosen due to its biocompatibility, high strength and low auto fluorescence. The two micro structured layers can be efficiently fabricated by hot embossing [4] and since the porous membrane is based on PC too, the components can be assembled by thermal bonding. The transparency of the whole system allows investigating the entire process of cancer cell extravasation by live cell and fluorescence microscopy.

For the characterization of the barrier properties of endothelial cells on the membrane a suitable sensor system is integrated. The impedance spectroscopy [5, 6] is an accurate method to examine the electrical properties of membranes or cell layers. The impedance, i.e. the AC impedance of a cell monolayer, represents a measure of the permeability of the monolayer relative inorganic and organic ions.

EXPERIMENTAL SETUP

For the biological experiments, the lower channel was filled with matrix components. Then human endothelial cells (EC) were seeded into the upper channel system. After 48 hours, an almost homogeneous monolayer of EC was achieved. After this time an isoosmolar solution containing green-stained cancer cells was flooded over the EC layer inside the upper channel system to investigate the migration steps of the cancer cells. It was observed that a small number of cancer cells penetrates the EC monolayer and the porous membrane and enters the matrix of the lower channel.

To perform impedance measurements, gold electrodes have to be structured on top of the porous membrane using optical lithography and chemical etching (cf. Figure 2). Finally an insulating layer has to be deposited on top of the whole membrane and structured lithographically, as shown in Figure 3. Currently, two different types of insulating material are tested: a standard photoresist AZ 4533 and polymethylmethacrylate (PMMA). For preliminary measurements the functionalized membrane is positioned on the bottom of a special test chamber with lateral feed through for the electrodes to be connected externally. The reservoir above the membrane is filled with phosphate buffered saline (PBS = phosphate buffered saline) as electrolyte. Later it can be used to cultivate endothelial cells. A Platinum wire in the cover plate of the test chamber is used as counter electrode. The whole setup is shown in Figure 4. The measurements are carried out in the high frequency range (700 Hz to 1 MHz) applying a multi-sine wave signal as a broadband stimulus signal. It is optimized so that the total energy

is within the selected frequency range. The signal to noise ratio was improved by averaging. The results of the impedance measurements of the individual electrodes are shown in Figure 5. As expected larger electrodes have a smaller impedance. The next step is the realisation of impedance measurements while cultivating living cells on top of the membrane.

Figure 6 shows the cross section of the planned microfluidic system, which contains gold electrodes on top of the porous membrane, to perform impedance measurements under flow conditions. The counter electrodes are located on top of the upper channels.

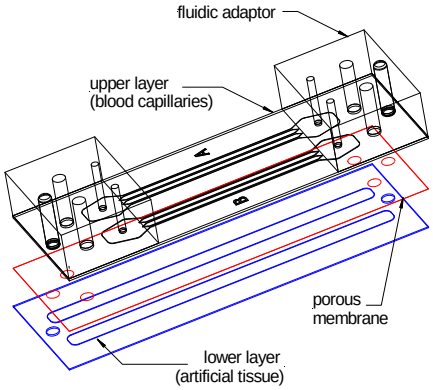

Figure 1: Schematic view of the artificial blood vessel system (exploded view).

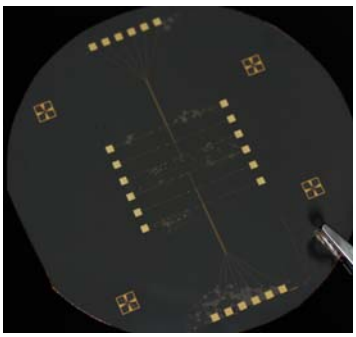

Figure 2: Photograph of the functionalized membrane with micro structured gold electrodes.

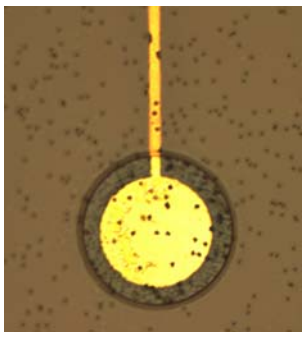

Figure 3: Photograph of a single electrode on top of the porous membrane with insulating layer.

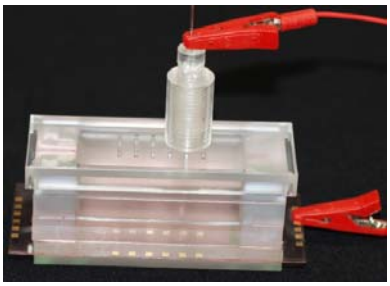

Figure 4: Photograph of the test setup with a Platinum wire as counter electrode.

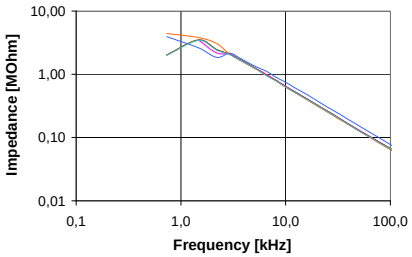

Figure 5: Impedance between the individual electrodes and the platinum counter electrode (the different colors correspond to the different electrodes).

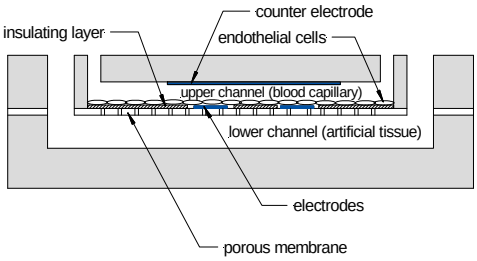

Figure 6: Schematic view (cross section) of the complete microfluidic chip with integrated electrodes for impedance measurements.

ACKNOWLEDGEMENTS

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