DESIGN OF A CELL-BASED LAB-ON-A-CHIP WITH INTEGRATED ELECTROCHEMICAL AND OPTICAL DETECTION

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Abstract – The Lab-on-a-Chip (LoC) described here is designed for the complex analysis of cytotoxic effects of chemical substances imposed on human keratinocytes. This LoC operating in real time will have promising applications in cosmetic and pharmaceutical industries. All parameters and conditions on the LoC were optimized with respect to practical aspects under semi-automated conditions. The system includes monitoring of the cells via microscopic supervision as well as detection of stress-induced green fluorescence protein (GFP) expression combined with an impedimetric readout for quantification of cell morphology.

Keywords: microfluidics, Lab-on-a-Chip, skin irritation tests

1. INTRODUCTION

For many purposes the development of a cell-based assay as part of a Lab-on-a-Chip (LoC) for the investigation of the cytotoxic effects of a chemical substance towards keratinocytes is aspired. The layout of the chip system, the integrated micro mixing strategy, the dimension of the cell culture chambers and the combination of electrochemical and optical readouts are critical factors in designing an *in vitro* assay enabling real-time, non-invasive and sensitive measurements. The design of the LoC is characterized by a high complexity and technical cross talk, which needs to be considered and combined to meet the demands of a semi-automated *in vitro* test system in addition to common cell culture practise.

Statistically confirmed results on live cell sensors are usually achieved by investigation of numerous cells, resulting in rather large cell culture chambers. In contrast a minimal consumption of cell culture media and chemical substances under test is required for the sake of cost reduction in a flow-through system. Furthermore, shear stress effects on the cells in the microfluidic channel need to be minimized. The applied flow rates have to be sufficiently low in order to guarantee minimal shear stress and low media consumption on the one hand, but a homogeneous and continuous supply with culture media and test substance on the other. Thus, it is important to realize an excellent mixing strategy at different flow rates. Moreover, the electrode design for electrochemical detection has to be optimized for sensitivity, stability and electric field distribution.

To meet all these demands the designed LoC has been optimized with respect to cell culture chamber size, flow rates as well as optimal sensitivity for the optical and the electrochemical readout.

2. CHIP DESIGN

The two-layer chip holds nine individually addressable cell culture chambers that allow a triplicate detection of the chemical under study together with the corresponding positive and negative controls. The media supply of cells via the flow-through chamber and the admixture of the analyte are guaranteed by micro mixers and the use of syringe pumps. The chip system is fabricated in cyclic olefin copolymer (COC), because of its high transmission for near UV and visible light, its excellent biocompatibility and its chemical as well as thermal stability up to 150 °C [1].

The chip system – manufactured in the same dimensions as a common microscopic slide – holds five inlets (Fig. 1). These allow the connection of the microfluidic devices via Luer-Locks. Three of these inlets are used for media supply whereas two additional ones are inserted in front of the micro mixer to ensure the application of the test substance as well as a positive control.

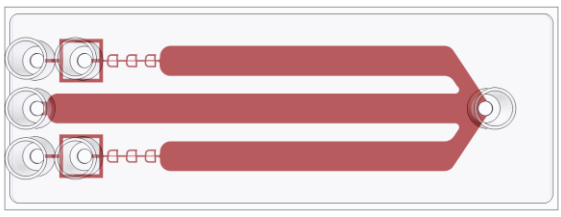

Fig. 1. Top view of the chip system. The chip system – manufactured in the same dimensions of a common microscopic slide – holds five inlets, two micro mixers for the test and reference substance and three parallel micro channels.

The cells are grown on the surface of small and planar gold-film electrodes, which are deposited on

the bottom of the cell culture chambers (Fig. 2). An integrated local heating device on the back side of the chip system enables cell growth independent a typical incubator.

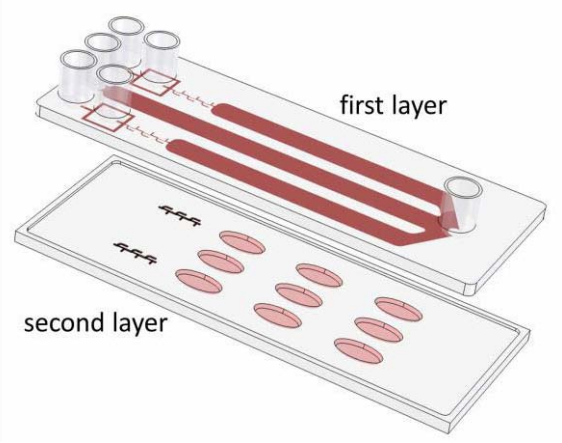

Fig. 2. Exploded view of the two layer chip system. The first layer holds the inlets and the flow through chamber. The second layer holds nine individually addressable cell culture chambers that allow a triplicate detection of the chemical under study together with the corresponding positive and negative controls.

2.1. Micro mixer

For optimal mixing of the chemical under study with cell culture media at very low flow rates (1 µl/min to 250 µl/min) a 'Tornado-Mixer' has been developed and characterized. By combining splitting, rotating and twisting elements (Fig. 3) the mixer requires only two micro-structured layers and already performs sufficiently well when arranged three times in series.

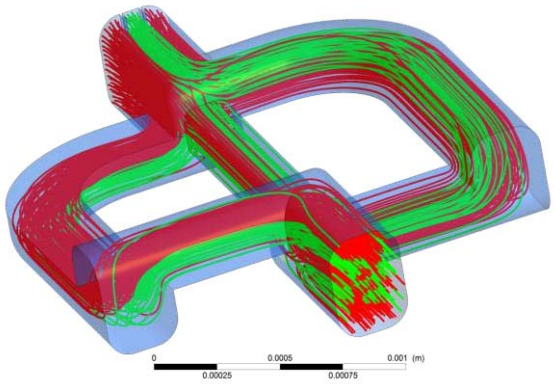

Fig. 3. Streamlines inside a single 'Tornado Mixer' module. The red flow lines represent the culture media, whereas the green ones visualize the chemical substance under study.

The arrangement of three serial linked micro mixer modules produces a good multi-lamination with an immense interface for mass transfer (Fig. 4).
The fluidic optimization of the 'Tornado Mixer' is based on numerical simulations by ANSYS CFX. Therefore a hybrid mesh with 50 million cells was used at a mixer volume of 1.42 µl. The used grid resolution is in the range of 1.5 µm, which is necessary for investigation of the diffusion effects at the interface between the two substances. The fluidic conditions are generally laminar and not transient.

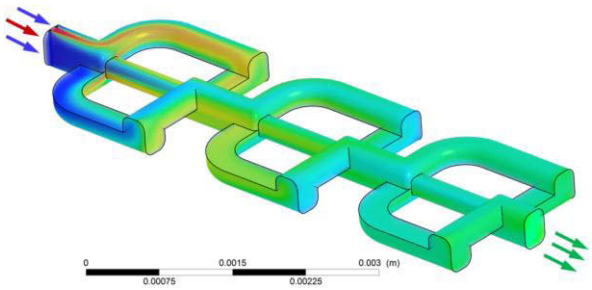

Fig. 4. 'Tornado Mixer' replicated three times in series. The flow profile of two different colored substances and their mixture is shown.

2.2. Cell culture chambers

For cell cultivation a trap based on mechanical and physical methods was designed directing special attention to the minimization of shear stress effects. A high shear stress rate behaves like an exogenous stimulus, inducing stress-activated cell signalling pathways. In order to reduce false-positive results the geometry of the cell culture chambers was optimized, based on numerical simulations by ANSYS CFX.

The shear stress on the bottom of the cell culture chamber mainly depends on the depth wells. Cells settle down when overflowing the cavity and adhere to the gold-film electrodes serving as culture substrate. As described before a maximum shear stress of 25e-03 kg/m·s^2 should not be exceeded in order to avoid negative effects on adherent cells [2]. Fig. 5 visualizes that the shear stress within the cell culture chamber reaches a maximum of 5e-06 kg/m·s^2 and, thus, remains beneath the critical value at the desired flow rates.

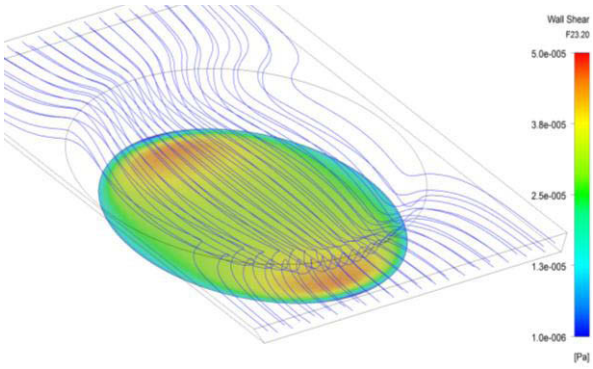

Fig. 5. Visualization of the shear stress at the bottom of the cell culture chambers. The blue flow lines visualize the flow conditions. Shear stress depends on the depth of the channel and the cell reservoir.

2.3. Electrochemical detection

Electrochemical detection of cytotoxic stress will be performed in two modes: impedance analysis and potentiometric measurements of the open-circuit potential (OCP). For both readouts the cells are grown on gold-film electrodes which are prepared by sputter coating / photolithography on the bottom of the flow chamber. Impedance analysis and OCP-measurements are essentially non-invasive and provide time-resolved information on any cytotoxic response of the cells. Previous studies have indicated that both methods are well-suited and highly sensitive to monitor cellular stress [3]. Whereas impedimetric monitoring reports on cell shape changes, OCP-readings provide supporting information about the interactions of the cells with the electrode surface.

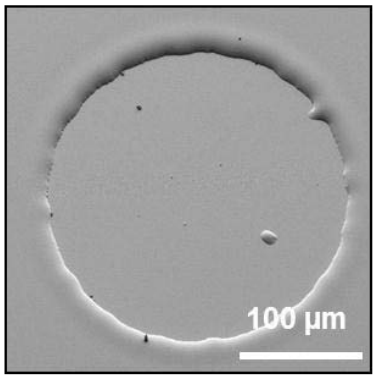

Fig. 6. Scanning electron micrograph of a gold-film electrode delineated by an insulating polymer.

The gold-film electrodes (Fig. 6) have a small surface area, providing highly sensitive impedimetric measurements of cells in a confluent monolayer residing on the electrode surface (Fig. 7).

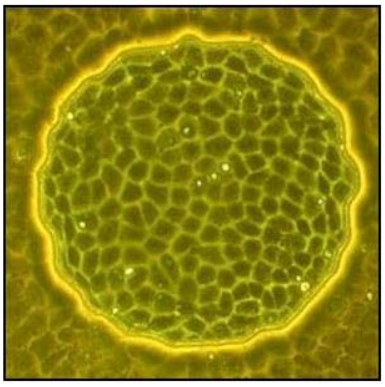

Fig. 7. Gold-film electrode covered with a confluent cell monolayer.

Fig. 8 presents the impedance spectrum for a cell-covered (red) and a cell-free gold-film electrode, immersed in cell culture medium (black). The presence of cells on the electrode surface causes the impedance to increase in a certain frequency range, since the cells act like insulating particles, forcing the current to flow around the cellular bodies.

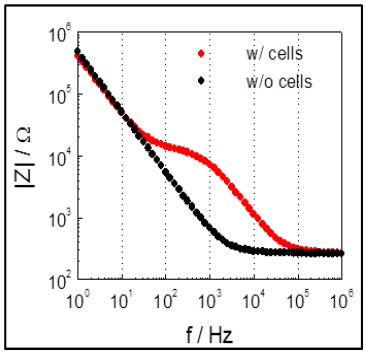

Fig. 8. Impedance spectrum of a cell-free (black symbols) and a cell-covered gold-film electrode (red symbols).

2.4. Optical detection

To provide an optical detection of the substance-related stress upon intracellular signalling cascades the used human keratinocyte cell line HaCaT [4] was stable transfected with a stress promoter-reporter-plasmid to create an intelligent sensor cell line. The promoter only induces the expression of the reporter gene after exposure to a toxic agent (Fig. 9). Hence, the optical readout of the fluorescence intensity in dependence on the cellular stress level is possible. The designed optical set up [5] realizes a microscopic monitoring of the cell culture specific cell density, cell adhesion and possible a contamination at the same time.

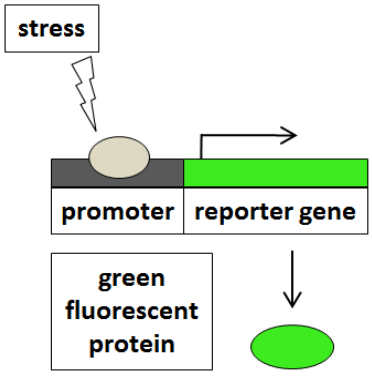

Fig. 9. Schematic of the genetic modification necessary to yield a stress sensitive GFP expression.

3. CONCLUSION

The aim of our work is the development of a cell-based lab-on-a-chip system to test the physiological or toxic effects of chemical substances imposed on human skin cells. The unique feature of our chip design is the combination of optical and electrochemical detection units as well as meeting the requirements of good cell culture practice.

By recording several cell parameters on chip it is possible to quantify cellular behaviour upon exposure to a certain test substance in a very complex and sensitive manner in real time, thereby allowing a detailed discrimination between competing, growth- and stress-related effects. This design provides an important basis for the development of micro-total analysis systems and can be applied to toxicology and drug screenings.

ACKNOWLEDGEMENTS

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