A CELL-BASED LAB-ON-A-CHIP AS AN ALTERNATIVE METHOD FOR TESTING SKIN IRRITATIONS

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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, Sensor cell line, Skin irritation tests

INTRODUCTION

The evaluation of the cell damaging potential of substances by animal testing is prohibited for cosmetics, and screening of many chemicals is required within the European Union regulation REACH. Thus urgent needs for predictive \textit{in vitro} assays exists [1]. Based on these facts the development of a cell-based Lab-on-a-Chip for the complex analysis of cytotoxic effects of chemical substances imposed on human keratinocytes is reported. The innovative method is described by the combination of optical and electrochemical readouts, which will be used as an alternative, and sensitive \textit{in vitro} assay.

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 \textit{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 \textit{in vitro} test system in addition to common cell culture practice. 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.

EXPERIMENTAL

Design of the Microfluidic System The three-layer chip (Figure 1) 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.

Figure 1: Layout of the three-layer chip system and the numerical simulation of a ‘Tornado-mixer’
The chip system – manufactured in the same dimensions as a common microscopic slide – holds five inlets. 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. 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. An integrated local heating device on the back side of the chip system enables cell growth independent of a typical incubator.

**Construction of the Sensor Cell Line** For the establishment of the *in vitro* assay the keratinocyte cell line HaCaT [2] was used. The cells were stable transfected with a plasmid, where a green fluorescent protein (GFP) and the heat shock protein A1A (HSPA1A) promoter are involved. The functional promoter region of HSPA1A was amplified by polymerase chain reaction and ligated into the pAcGFP1.1 vector (Takara Bio Europe/Clontech). The constructed plasmid was transfected into the cells using TurboFect™ (Fermentas). Stable cell lines were obtained by selection with 1 mg/ml G418. Resulting colonies that showed the maximum fluorescence response was subcloned, leading to the establishment of sensor cells.

**RESULTS AND DISCUSSION**

**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 the mixer requires only two micro-structured layers and already performs sufficiently well when arranged three times in series producing a good multi-lamination with an immense interface for mass transfer (Figure 1). 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.

**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 signaling pathways. In order to reduce false-positive results the geometry of the cell culture chambers was optimized. 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⁻⁰⁵ kg/m·s⁻² should not be exceeded in order to avoid negative effects on adherent cells [3]. Numerical simulations by ANSYS CFX visualized 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.

**Optical Detection** To provide an optical detection of the substance-related stress upon intracellular signaling cascades the used human keratinocyte cell line HaCaT was stable transfected with a stress promoter-reporter-plasmid to create an intelligent sensor cell line. Recent research has mainly focused on HSPA1A that is expressed in human keratinocytes. High expression levels of this protein protect cells from further stress exposure. The capability of that biomarker was analyzed by quantitative real-time PCR. The HaCaT cells were incubated under the following conditions: heat shock (Figure 2A) and exposure to cadmium chloride (Figure 2B) to detect the relative gene expression (2还没ΔΔCt) of HSPA1A. The data showed a high gene expression of HSPA1A after 2 h of heat shock and incubation with cadmium chloride for 6 to 8 h.

![Figure 2](image)

Figure 2: Detection of the relative gene expression (2还没ΔΔCt) of HSPA1A after heat shock (43 °C; 0, 1, 2 h) (A) and incubation with cadmium chloride (25 µM; 0, 1, 3, 6, 7, 8 h) (B) [control vs. sample = n.s. - not significant; * / ** / *** - significant]

Based on these results a cytotoxic sensor cell line was established. Figure 3 illustrates that the used promoter region is able to up-regulate the expression of GFP following the exposure to cadmium chloride in comparison to a control population. Hence, the optical readout of the fluorescence intensity in dependence on the cellular stress level is possible. The designed optical set up [4] realizes a microscopic monitoring of the cell culture specific cell density, cell adhesion and a possible contamination at the same time.
Development of an integrated microenvironment to study cells with the electrode surface. By recording several cell parameters on chip it is possible to quantify cellular behavior 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-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.

CONCLUSION

The aim of our work is the development of a cell-based lab-on-a-chip system to test the toxic effects of chemicals imposed on human skin cells. The unique feature of our chip design is the combination of optical and electrochemical readouts as well as meeting the requirements of good cell culture practice. By recording several cell parameters on chip it is possible to quantify cellular behavior 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-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

This study is supported by the German Federal Ministry of Economy and Technology (BMWi) within the ZIM program.

REFERENCES


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