MONO-, CO- AND MIXED CULTURE OF CELLS IN THE MICROSYSTEM FOR PHOTODYNAMIC THERAPY PROCEDURES
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ABSTRACT
In this paper, we present the evaluation of PDT procedures in the microsystem, in which three kinds of cell cultures (mono-, coculture and mixed culture) were performed, simultaneously. In contrast to our earlier work the geometry of the microsystem was improved: three pairs of additional microchambers for monoculture of cells were added and the microsystem was enriched of concentration gradient generator (CGG). The geometry of the microsystem allowed for introducing separately two cell lines (human lung carcinoma - A549 and normal mouse embryo - Balb/3T3) into the microchambers.

KEYWORDS: monoculture, coculture, migration, microfluidic system, photodynamic therapy (PDT)

INTRODUCTION
Developing of biologically relevant models of human tissues and organs is an important step for modeling disease or drug discovery. Microsystems are functional units of such models, which are used for analysis of cell-cell interactions and migrations [1,2]. A microsystem can also be used for examination of photodynamic therapy (PDT) procedures. However, only a few scientific groups investigated PDT procedures in microscale. Xia Lou presented a microchip for high- throughput PDT screening, in which parameters on cells monoculture were analyzed [3]. There are various methods for analysis of viability of cells. Most often, staining with calcein AM and propidium iodide is used. In turn, supernatant taken from cells cultured in a microchip is measured using multiwell plates. Therefore, we also proposed the microsystem for analysis of cells’ supernatant, taken from the microsystem for PDT procedures.

EXPERIMENTAL
The (PDMS/glass) microdevice for PDT procedures consists of four microstructures with a network of microchannels and eight pairs of culture microchambers. The arrangement of the microchannels’ network on the plate creates a V-shaped structure with three pairs of microchambers without connecting microchannel and five pairs of microchambers connected with additional microchannels (a width of 100 µm, a length from 200 µm to 1000 µm). At the end of each microstructure, the common microchamber was placed (Figure 1). The microsystem consists also CGG, which enabled to obtain four different concentrations of a photosensitizer in a single step.

![Figure 1: (A) The geometry of the microfluidic system used for evaluation of PDT procedures. Four separate microstructures were connected with CGG. (B) The microstructure with microchambers for mono-, co- and mixed culture area. Simulation of 2µl/min flow rate was marked by red and blue colors.](image-url)
RESULTS AND DISCUSSION

In Figure 2 the fabricated microsystem is shown. The microchannels’ geometry ensured proper flow of cells’ suspension or substances and allowed for simultaneous introducing of two cell lines Balb/3T3 (normal mouse embryo) and A549 (human lung carcinoma) into separate microchambers.

![Figure 2: The fabricated microsystem for evaluation of PDT procedures.](image)

After introduction of the cells, in each microstructure three kinds of cultures were obtained: monoculture (first three pairs of microchambers), coculture (five pairs of microchambers connected with various length of microchannel) and mixed culture in the common microchamber (Figure 3).

![Figure 3: Microchambers with A549 and Balb/c 3T3 cells cultured as a monoculture, coculture and mixed culture.](image)

Growth and cells proliferation was observed in the microsystem for 48 hours. After this time, we studied how PDT procedures influence on the viability of Balb/c 3T3 and A549 cells (parameters of PDT were elaborated in our previous work [4]). In this purpose, through CGG inlets 0 mM and 0.75mM of 5-aminolevulinic acid (ALA) were introduced. Thus, in each microstructure a different concentration of the photosensitizer was obtained. After PDT procedures, Balb/c 3T3 cells were still alive almost in all microchambers (Figure 4).

The lowest viability of the Balb/c 3T3 cells was observed in the microchannel with 300 µm long connecting microchannel (76.6% for 0.5 mM and 79.2% for 0.75 mM) and the common microchamber (82.6% for 0.75 mM). Probably, the death factors which were produced by the A549 cells have toxic effect also on normal cells cultured in microchambers placed in closer distances. Moreover, we proved that the number of A549 (carcinoma) dead cells was dependent on the ALA concentration (Figure 5).
The microsystem for evaluation of PDT procedures, can also be connected to different microsystem, in which analysis of microvolumes of cells’ supernatant can be performed (Figure 6). This microchip combines spectrophotometer-based detection, real-time signal processing and indirect determination of supernatant i.e. LDH activity analysis.

**CONCLUSION**

In the microsystem (for PDT procedures) three kinds of cell cultures (mono-, coculture and mixed culture) were performed, simultaneously. The obtained results confirmed that the microsystem can be useful tool for evaluation how the presence of another cell type have influence on the cells viability after PDT procedures. Our further studies will be focused on testing PDT procedures also through LDH activity analysis (using the microsystem for analysis of cells’ supernatant).

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