PHOTODYNAMIC THERAPY PROCEDURES ON LUNG CARCINOMA AND NORMAL CELLS COCULTURE IN THE MICROFLUIDIC SYSTEM

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ABSTRACT

In this paper, we describe the evaluation of PDT procedures performed in a newly designed microsystem in which migration and interaction between normal and carcinoma cells are possible. The geometry of the microsystem contains two microstructure with a network of microchannels and five pair of culture microchambers. Each pair is connected with an additional channel. The geometry of the microsystem allowed for introducing separately two cell lines (human lung carcinoma - A549 and normal - MRC-5 cells) into the microchambers. Moreover, growth and migration of the cells were monitored. Finally, in this coculture the toxic effect after PDT procedures was investigated.

KEYWORDS: normal-carcinoma cells coculture, migration, microfluidic system, photodynamic therapy (PDT), 5-aminolevulinic acid (ALA)

INTRODUCTION

The development of novel anticancer drugs or therapeutic approaches is related with knowledge of cellular functions. Carcinoma cells grow in the place and in direct contact with normal cells. Therefore, understanding of cell migration at the molecular level and normal-carcinoma cells interactions cultured in the same environment/medium is very important [1]. Photodynamic therapy (PDT) is a promising method for the detection and treatment of cancer, which must be still examined. The cells migration analysis or investigation of PDT procedures can be performed in microfluidic systems [2,3]. The application of microdevices allows for investigation in more realistic cell-cell interaction than in a classic cell culture. Moreover, fast and cheap evaluation of cytotoxicity and anticancer therapies is possible.

EXPERIMENTAL

The designed (PDMS/glass) microdevice consists two microstructures with a network of microchannels and five pair of culture microchambers. Each pair is connected with connecting microchannel (a length from 30µm to 300µm). At the end of channels the common microchamber was placed (Figure 1).

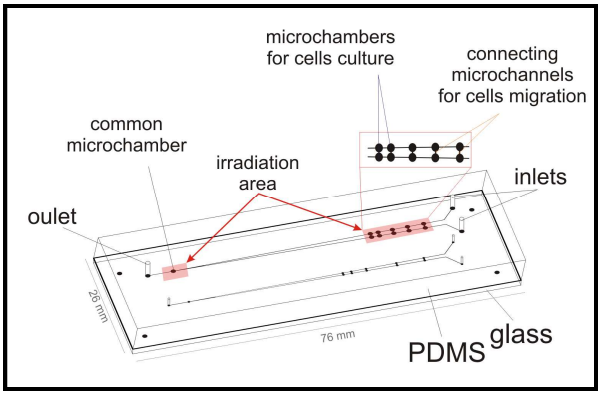

Figure 1. The geometry of the microfluidic system used for evaluation of PDT procedures, a length of connecting microchannels was 300, 200, 100, 80 and 30 µm respectively. Two separate microstructures were fabricated with different dimension of microchambers (1 mm and 500 µm).

In order to evaluate the effectiveness of PDT, human lung carcinoma-A549 and normal-MRC-5 were seeded in appropriate microchambers without affecting each other. Parameters of PDT procedure (with 5-aminolevulinic acid) were elaborated in our previous work (irradiation trough the PDMS cover using a high power LED: λ=625nm, t=60s, energy dose=30J/cm^2) [3].

RESULTS AND DISCUSSION

In figure 2, MRC-5 and A549 cells after introduction in the microchambers are shown. The last microchamber is common for both cell lines. The microchannels' geometry ensured proper flow of cells' suspension and allowed for simultaneous introducing of two cell lines into separate microchambers. Moreover, connecting microchannels enabled migration of the cells and medium exchanging from two cultures. After 24 h, both of cell lines very well adhered to growth surface. We ob-

served that only isolated MRC5 cells migrated in the specially fabricated connecting channels. It has been found that the shorter distance between microchambers, the faster cell migration. Figure 3 shows the microchambers with two different cell lines A549 and MRC5(a length of connecting microchannels -30 µm).

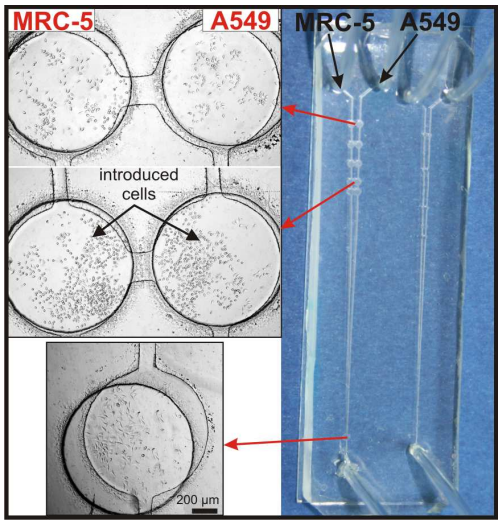

Figure 2. The MRC-5 and A549 cells cultured in the microchambers with connecting microchannels (300 and 100 µm) for migration analysis. The last microchamber is common for both cell lines.

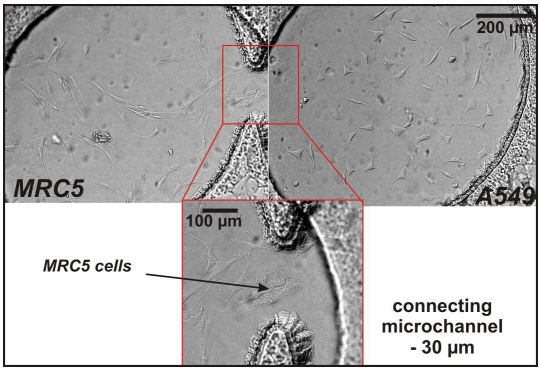

Figure 3. The MRC-5 and A549 cells cultured in the microchambers with 30 µm connecting microchannels for migration analysis.

The toxic effect after PDT procedures (with ALA) on the separated MRC-5 and A549 cell culture was investigated. It was proved that the usage of 0.75mM ALA in PDT procedures caused death of 95% of carcinoma A549 cell, whereas only 5% of normal MRC-5 cells. Next, we studied how the migration of the cells in the connecting microchannels and medium exchanging in these connecting microchannels influence on the viability of the MRC-5 and A549 cells after PDT procedures.

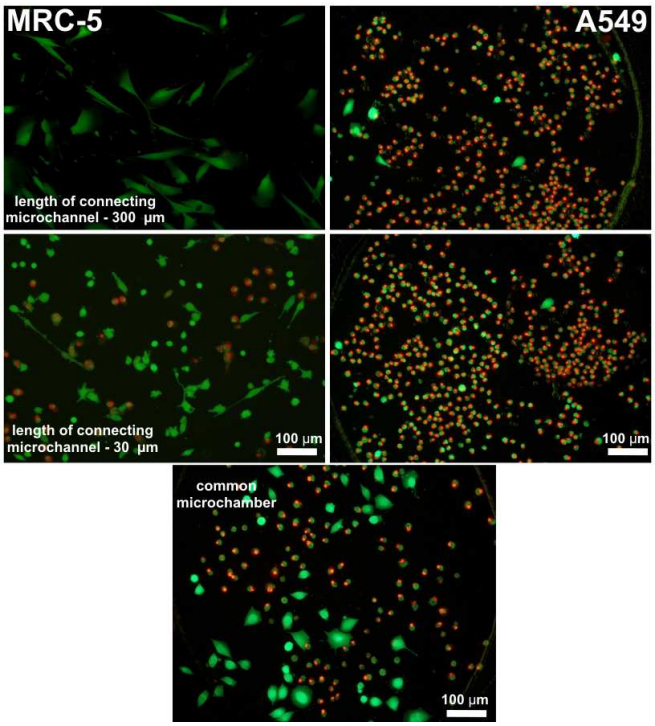

Figure 4. The MRC-5 and A549 cells cultured in the microchambers after PDT procedures and viability test with calceine AM and propidium iodide.

72 hours after MRC-5 and A549 cells seeding, the PDT procedures with 0.75mM ALA in the microsystem were performed (Figure 4). After viability test (with calceine–AM and propidium iodide) MRC-5 cells were still alive and A549 cells were dead in the pair of microchambers connected with microchannels with a length of 80 – 300 µm. We observed that, the number of the dead normal cells in the shortest connecting microchannel (30 µm) and the common microchamber was the highest (Figure 5). Probably, reactive oxygen species which were produced by the A549 cells have toxic effect also on normal cells cultured in the microchambers placed in closer distances.

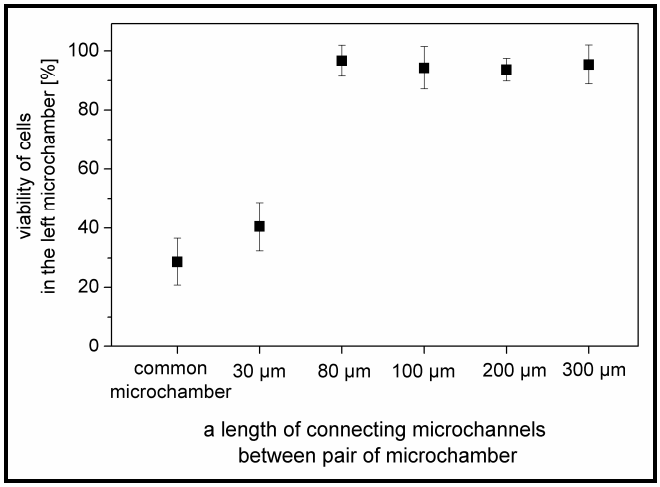

Figure 5. The number of the dead MRC-5 cells (normal) in the each microchambers

CONCLUSION

The obtained results confirmed that the microsystem can be a useful tool for cells migrations analysis and evaluation how the presence of another cell type have influence on cells viability after PDT procedures. The microsystem can be applied for cytotoxicity analysis of potential anticancer compounds. Our further studies will be focused on testing PDT procedures for different values of PDT parameters. Our results will be useful for further biological and medical researches. Application of microsystem can be helpful during optimization of clinical PDT parameters (*i.e.* the dose of irradiation, time of exposition, concentration of photosensitizers).

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