Microfluidic devices — application in anticancer studies

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A rapidly growing pharmaceutical industry requires faster and more efficient ways to find and test new drugs. One of the new method for cell culture and examining the toxic effects of drugs is application of microfluidic systems. They provide new types of microenvironments and new methods for investigation of anticancer therapy. The use of microsystems is a solution that gives the opportunity to reduce not only cost and time, but also a number of tests on animals. In this paper we present designed and fabricated hybrid microfluidic systems which are applicable for cell culture, cell based cytotoxicity assays and photodynamic therapy procedures. Polydimethylsiloxane (PDMS) and sodium glass were used for fabrication of microdevices. The designed geometry of the microdevices includes cell culture microchambers and a concentration gradient generator (CGG). The CGG enables to obtain different concentrations of tested drugs in a single step, which is a significant simplification of cytotoxicity assay procedure. In the designed microsystems three various cell lines (normal and carcinoma) were cultured and analyzed.

Keywords and phrases: microfluidic system, PDMS, adherent cell culture, concentration gradient generator (CGG), cytotoxicity tests, photodynamic therapy (PDT) procedures.

Introduction

Cancers are today the most common diseases in the world. In recent years the number of cancer patients is still increasing. Lifestyle of modern civilization and the relatively late diagnosis makes these statistics even increasing. In medicine, various types of cancer therapy are used: chemotherapy, radiotherapy, surgery, phototherapy [1].

The chemotherapy is one of the anticancer method in which a cytostatic drugs are used. Most commonly, chemotherapy acts by killing cells that divide rapidly, one of the main properties of most cancer cells. However it has also toxic effect on the normal cells that divide rapidly under normal conditions [2]. Multidrug chemotherapy is also used in medicine, because it combines two or more different agents simultaneously in order to enhance their effectiveness. The aim of this method is to obtain not only additive but especially synergistic effect of investigated agents [3]. In the photodynamic therapy (PDT) three factors are used simultaneously: a photosensitizer (for example: 5-aminolevulinic acid — ALA), light of a wavelength that is absorbed by the photosensitizer, and intracellular oxygen. The specific wavelength induces a photosensitizer accumulated in carcinoma cells. Next, the induced photosensitizer reacts with oxygen present in the cells and produces reactive oxygen species (ROS), which cause death of tumour cells [4]. The concentration of PPIX (accumulates in the cells after introduction of exogenous ALA) in the tumor cells is lower than in the normal cells, because the enzyme activity in the carcinoma cells, which converts PPIX to heme has been found to be reduced [5].

The utilization of microsystems is a new method which enables examination and investigation of anticancer therapies. The usage of the miniature analytical systems assures small consumption of solvents, short reaction times, portability and low cost. The microfluidic devices are playing an important role, because they create more realistic cell-cell interaction than in a classic cell culture. In the microdevices it is possible to create conditions for cell growth, migration and adhesion. The usage of the microdevices allows for a faster and cheaper evaluation anticancer procedure such as cytotoxicity of drugs or photodynamic

therapy procedures for various cell lines. The aim of our research was to design and to fabricate hybrid microfluidic systems which are applicable for cell culture, cell based cytotoxicity assays and photodynamic therapy procedures.

Experiments

Microfluidic devices

We developed two microsystems dedicated for cell analysis (Fig. 1). PDMS and glass were chosen as materials used to fabricate the microsystems. PDMS is nontoxic, biocompatible and permeable to gases, so exchange of gases between culture microevrinoment and incubation air is possible. However, the hydrophobic properties of the PDMS do not enable adhesion of adherent cells, so the places of cells' growth must be hydrophilic. Therefore microchambers (a diameter of 1mm and a depth of 30μm) for cell culture were fabricated (by wet etching) in the hydrophilic glass plate. Both of used materials were transparent, so real time monitoring of the cells proliferation and viability tests were possible. The microchannels (a width of 100 μm and a depth of 50 μm) in the PDMS were fabricated by soft lithography and replica molding techniques. The network of microchanels creates concentration gradient generator (CGG) included two inlets.

Cells culture

In the sterilized microsystems cells suspensions density (1×10^6 cells/ml) were introduced using syringe pump (at a flow rate 20 μl/min). The various cell lines were cultured and tested in the microsystems: human lung carcinoma (A549), human colon carcinoma (HT-29), and normal mouse embryo (Balb/3T3) cell lines. In Fig. 2, the morphology of A549, Balb/3T3 and HT-29 cells cultured in the microdevices is shown. We observed that the morphology of the tested cells is a bit different. Balb/3T3 and A549 steadily colonize the growth surface, but the normal cells are larger and have thinner membrane than carcinoma cells. HT-29 cells create characteristic clusters. Besides of these differences the gene expression is also different. The optimized conditions in the microsystem allowed the adhesion and proliferation of all tested cell lines. The geometry of the microsystem and the applied procedures did not cause hydrodynamic stress to the cells cultured in the microchambers.

Results

In the designed microsystems PDT procedures and cytotoxicity tests were performed. In the microsystem 1 the toxic effect of 5-fluorouracil (5-FU) was tested on the A549 and HT-29 cells. Through the CGG inlets at a flow rate of 1.2 μl/min for 50 min two different solutions of the 5-FU (0 and 300 μM) were introduced. Consecutive series of culture microchambers achieved 5-FU concentration of 0, 75, 150, 225 and 300 μM. The viability tests (using calceine AM and propidium idiode) were

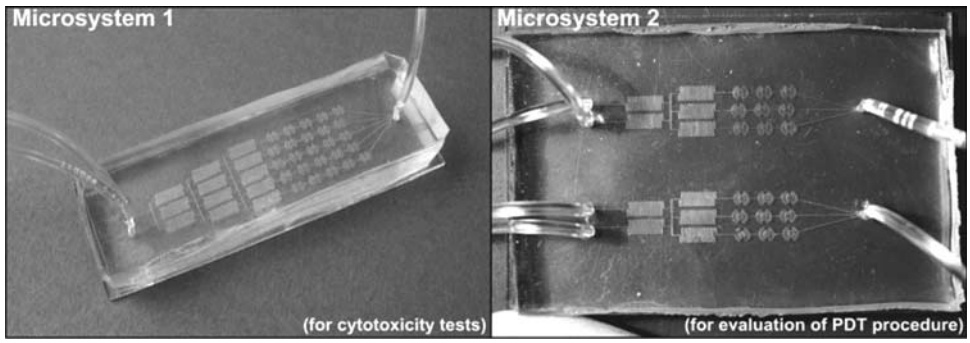

Fig. 1. The PDMS/glass microsystems with concentration gradient generator (CGG) (A) for cytotoxicity tests (B) for evaluation of PDT procedure.

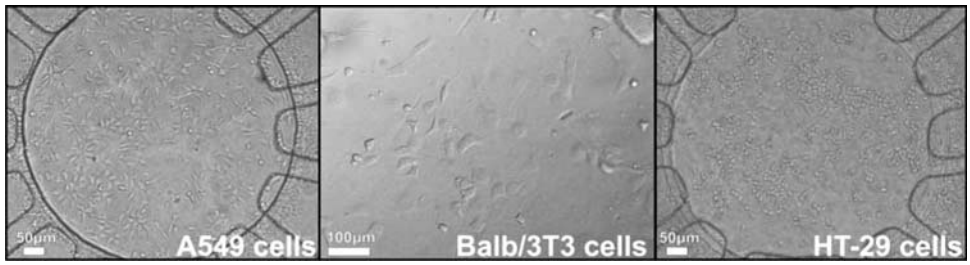

Fig. 2. The microchambers with culture of (A) A549 cells (B) Balb/3T3 cells (C) HT-29 cells.

determined 24 and 48 h after drug introduction. The number of dead cells after incubation with 5-FU increases with the drug concentration (Fig. 3). 5-FU tested at concentrations ranging from 0 to 300 μM, inhibited the survival of both cell types. However, HT-29 cells were less sensitive to the cytotoxic agent than A549 cells in both incubation times (*i.e.* 24h and 48h). The specific morphology of two different cell lines may be one of the reasons for these differences.

drug concentration [μM]	A549 24 h	A549 48 h	HT-29 24 h	HT-29 48 h
0	94.6	94.0	97.5	97.2
75	78.9	69.6	88.9	80.0
150	70.2	48.8	87.9	75.4
225	47.7	38.2	85.4	73.2
300	37.9	26.2	76.2	64.0

Fig. 3. The viability [%] of A549 and HT-29 cells after incubation with 5-FU.

The PDT procedure was performed in the microsystem 2. The A549 (carcinoma) and Balb/3T3 (normal) cells were cultured in the two structures of microsystem. The exogenous 5-aminolevulinic acid (ALA) was introduced into the microchambers at a flow rate of 1.2 μl/min through CGG. Three different concentrations of ALA were obtained (0, 0.375, 0.75 mM) in CGG outlets. After 4 hours of incubation, the cells were irradiated through the PDMS cover using a high power LED (λ = 625 nm, t = 60 s, energy dose = 30 J/cm^2). Viability of the cells was determined using an inverted fluorescence microscope in the presence of calceine AM and propidium idiode. Observed toxic effect of PDT procedures was higher for carcinoma A549 than for normal Balb/3T3 cells. For example, for the highest concentration of ALA introduced into microchamber the percentage of living cells was 19,5% for Balb/3T3, whereas for A549 cells 94%. For both cell lines the number of dead cells was increasing with higher concentration of ALA. In Fig. 4 the viability of A549 cells 24h after PDT procedure is shown.

Conclusions

The results show the possibility to perform the cytotoxicity tests and photodynamic therapy procedures in microscale. Application of this type of microfluidic devices is expected to have a significant influence on biological and engineering studies. The microsystems enable to perform fast and cheap tests with various kinds of drugs and cell lines. It can be a user-friendly device applicable in biological laboratory. In our opinion, such tests should be helpful during optimization of real clinical photodynamic therapy parameters (*i.e.* the dose of irradiation, time of exposition, concentration of photosensitizers).

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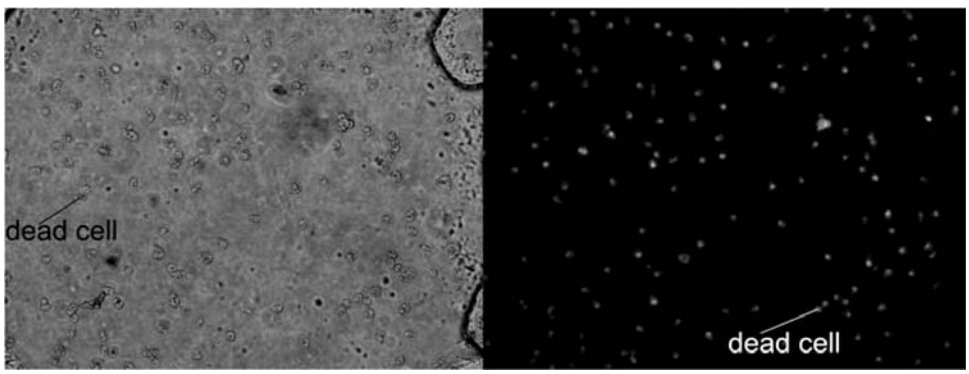

Fig. 4. The microchamber with A549 cells 24 hours after PDT procedure for 0.75 mM ALA.