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PDMS/glass microfluidic cell culture system for cytotoxicity tests and cells passage

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ABSTRACT

In this paper, hybrid (PDMS/glass) microfluidic cell culture system (MCCS) integrated with the concentration gradient generator (CGG) is presented. PDMS gas permeability enabled cells' respiration in the fabricated microdevices and excellent glass hydrophilicity allowed successful cells' seeding. The human lung carcinoma cells (A549) were cultured in the microdevice for several days. The growth and proliferation of cells was monitored using an inverted fluorescence microscope. After the cells' confluence was achieved in the microchambers, the novel method of cells' passaging in the designed microdevice was developed and successfully tested. The MCCS microdevice is fully reusable, i.e. it can be used several times for various cell culture and cytotoxic experiments. The suitability of designed MCCS for cell-based cytotoxicity assay application was verified using 1,4-dioxane as a model toxic agent. The series of cytotoxicity tests in the microdevice as well as in classic way using 96-well cell culture plates were performed to compare results obtained in micro- and macroscale. Fluorescein dibutyrate (FDB) and iodide propidine (PI) were used as viable and dead cells' markers, respectively.

Fabricated MCCS microdevices were reproducible and apart from cell culture for long period of time, including cell passaging, it allowed cell-based cytotoxicity assays performance. The MCCS can be applied in high-throughput cell-based assays providing important informations on potential drug targets, substances' bioactivity, defining the lowest toxic level of tested substances etc.

1. Introduction

Miniaturization is a new field of scientific research and engineering development. A micro-total analysis system (μTAS), also called "lab-on-a-chip", integrates many chemical operations on a single microchip. The microdevices are often applied for various biological tests [1]. Miniaturization has started to play an important role in genomics and proteomics, clinical diagnostic in medical sciences, pharmacology, tissue engineering and cell biology [2].

Cell engineering enables cells physiology study and has wide applications in many other fields of research, for example in advanced cell biology, medicine and toxicology [3]. Miniaturized versions of bioassays offer many advantages such as small consumption of solvents, reagents and cells, which is critical for valuable, available in limited volume, samples and for high-throughput screening. Moreover, they provide short reaction times, portability, low cost, low power consumption, versatility in design that leads to further integration with other miniaturized devices [4].

The microsystems dedicated for cells engineering should assure optimal growth of cells, control of cells migration, adhesion and should also provide optimal oxygenation and flow of culture medium [2,5]. Poly(dimethylsiloxane) (PDMS) is a material which is most often used for fabrication of microdevices for cell culture and cell-based assays [6,7]. Some hybrid microdevices (i.e. PDMS/glass) were also fabricated, e.g. a microsystem dedicated for growing and monitoring of cell lines and testing their exposure to drug or toxine [5,8].

The microdevices for cells engineering were used for single cell analyses [9–11], sorting [11,12], handling [13,14], lysis [11] and culture [5,6,8,10,15–17]. Many different types of cells lines were used in microsystems: hepatocytes [18], cardiomiocytes [19], fibroblast cell line [5], lung cells [20] and endothelial [21]. The microfluidic silicon/glass devices dedicated for cells immobilization and monitoring of the apoptotic process were fabricated [22]. Human promyelocytic leukemic cells (HL60) were trapped and apoptic cell death dynamics was measured on a chip in real time. The cell death was visualized and observed using microscopy. Leclerc et al. [15] described the fabrication of microdevices composed of two PDMS

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plates, which were successfully used for HEP G2 (hepatocarcinoma liver cells) cell culture. Two types of experiments were performed in the system: in the first one culture medium was changed every day and in the second medium was not changed at all. In the first case, successful culture could be performed for 10 days. Hung et al. [8] described a PDMS–glass microdevice containing a chamber for cell culture, where human carcinoma (HeLa) was cultured to the confluence state achievement. Different geometries of the microdevices were tested: containing central cell chamber, sieve with a separate channel for seeding or miniature traps [5]. In these microsystems, three cell lines were tested: fibroblast, Chinese hamster ovary cell (CHO) and hepatocytes, and their growth was monitored in 1–5 days following seeding. Cell culture and proliferation was obtained in all designed microdevices, but seeding density could not have been too low.

Although successful cell culture in microfluidic devices is widely described, the authors of the paper found only one communication on cells passage performed in microdevices [23]. The described passage was performed by trypsin perfusion through the microsystem. However this passage could be considered as partial because cells remained adherent in the microchambers, while classic cell passage includes cells detachment and following adhesion. Cell passage is an essential step for long-time cell culturing. It assures continuous proper growth and proliferation of cells [3]. Without cell passage, death of cells can be caused by decreasing of nutrient amount in the culture medium and increasing of toxic metabolism products. Moreover, adherent cells culture can be continued to the confluence state achievement, which occurs in several days after seeding. Lack of cell passage step leads to cell culture degeneration. Without cell passage step, microfluidic devices with cell culture are suitable for observation only for several days. Moreover, micro-scale observation of influence of numbers of cells' passages on alterations in cell morphology, chemotaxis, growth rate, protein expression and cell analysis can be performed.

In this paper, the hybrid microfluidic cell culture system (MCCS) integrated with the concentration gradient generator (CGG) was described. The designed microdevice was applicable for long-term cell culture, including cell passages, and for cell-based cytotoxicity assay. Application of microchambers etched in glass proved to be a good solution for adherent cells culturing. The designed microdevice is fully reusable, i.e. it can be used several times for various cell culture and cytotoxic experiments. There are almost no publications describing attempts of reusing such systems. The A549 human lung carcinoma cells were cultured in the microdevice for several days. The growth and proliferation was possible due to development of: (I) sterility, (II) cell seeding in MCCS and (III) medium dosage protocols. After the confluence state was achieved, the successful cell passage was performed inside our MCCS and up to now such an operation in a microdevice has not been yet described in the literature. The suitability of our microdevice for cell-based cytotoxicity assay application was verified using 1,4-dioxane as a model toxic agent. We performed the series of cytotoxicity tests in the microdevice as well as in classic way using 96-well cell culture plates to compare micro and macroscale results.

2. Design

2.1. Materials for the hybrid MCCS fabrication

A microsystem dedicated for cells engineering should assure optimal growth of cells. Therefore the materials, which we used for the microsystem fabrication, were biocompatible and non-cytotoxic. We decided to design and fabricate a hybrid microsystem made of two materials. The microsystem dedicated to cells engineering consisted of two plates. A plate containing CGG and microchannels providing medium flow were fabricated using poly(dimethylsiloxane) (Dow Corning Sylgrad 184). Second plate with a matrix of microchambers for cell loading and cell culture was fabricated using sodium glass (75 mm × 25 mm × 1 mm) (Helmand). The chosen materials are transparent, so the real time monitoring and analysis of living cells morphology is possible. Moreover, due to PDMS gas permeability an equilibrium state between the atmosphere present in the cell culture incubator and medium present in the microchambers was obtained and it enabled successful cells' growth in the microdevice. On the other hand, glass is a hydrophilic material and provides good adhesion of cells.

2.2. Microdevice geometry

The design of presented microdevice was based on the designs described by Hung et al. [8,23]. The construction of the device includes culture chambers (a diameter of 1 mm and a depth of 30 μm) and the concentration gradient generator. Adherent cells were seeded in the culture chambers and the mixture of model toxic substance (1,4-dioxane) and medium was dosed through the CCG to determine cytotoxicity of the substance. The geometry of microchannels and microchambers was designed using the AutoCAD software. In Fig. 1A, the geometry of microchannels (100 μm wide and 50 μm deep) in the PDMS layer is presented. The geometry of glass plate pattern contains the matrix of round microchambers in places corresponding to cell culture microchambers fabricated in the PDMS layer.

The concentration gradient generator (see Fig. 1A) has a structure with two inlets and five outlets [24]. Due to difficulty of good mixing in the microchannels we decided to introduce meander-shaped microchannels modules to the CGG design. The length of microchannel in one meander module was 50 mm, it was enough for effective fluids mixing by the diffusion. On every outlet of the CGG, the series of five culture chambers was situated. In the cytotoxicity assay, model toxic substance solution is diluted in the culture medium by the CGG to achieve five different concentrations that influence cells cultured in the chambers.

The culture chambers in the MCCS are designed for adherent cells culture cultivation (Fig. 1B). They were designed to assure the place for docking of introduced in MCCS of cells. The chambers etched in glass (a diameter of 1 mm, a depth of 30 μm) provided good cells adhesion conditions and minimized a hydrodynamic stress caused by the medium flow over the cell culture. The pattern of the microchannels around the culture chamber was designed to provide homogenous medium dosage. The results of medium flow simulations performed using COMSOL Multiphisics 3.3 are presented in Fig. 2. The medium flow visualization in the designed cell culture microchamber showed perfect medium dosing to the whole culture present in the chamber. It was found that this perfect dosing of the medium was independent on flow rates applied. Next, hydrodynamic conditions' influence on cells seeded in the microchamber was investigated and optimized.

3. Experimental

3.1. A hybrid microsystem fabrication: microchannels in PDMS

The microchannels and microchambers were fabricated in PDMS using photolithography and replica molding technique [25]. The capillary film (Pro/Cap 50, Chromaline) was deposited on sodium glass plate (75 mm × 25 mm × 1 mm) and was exposed to UV light through a photomask. The exposed photoresist was developed using water stream and was dried using nitrogen. In this step, we obtained a so-called stamp (for the replication in PDMS) which contained the microchannels' network and the areas for cell culture.