A USER-FRIENDLY, SELF-CONTAINED, PROGRAMMABLE MICROFLUIDIC CELL CULTURE SYSTEM FOR HIGH QUALITY MICROSCOPY

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ABSTRACT

We present a fully self-contained system for long-term cell culture with real-time, high quality microscopic access and programming capabilities for constant perfusion live-cell assays. When assembled, the system constitutes a closed unit complete with microfluidic culturing chip, reservoirs, interconnections and flow control without the need for external mechanical components. Being designed especially for use in cell labs and to fit into standard motorized stages in inverted life science microscopes the system is user-friendly for operators without a microfluidic background. To demonstrate the system capabilities we show examples of switching between different liquids and long-term cell culture with time-lapse imaging.

KEYWORDS: Programmable cell culture, Microscopy, Interconnection, Fluid actuation

INTRODUCTION

The use of microfluidic tools in cell culture is often limited by the complexity and need for peripheral components in typical research setups incompatible with standard, sterile cell biology work flows and microscopy [1,2]. This can be particularly pronounced in assays requiring high parallelization or multiplexed operation where external fluid actuation requiring e.g. large amounts of tubing from syringe pumps or pressure controllers can lead to unwanted dead volumes and portability issues thus reducing their suitability for routine work in a laboratory environment. A common way to overcome issues with peripheral components is to integrate fluid actuation directly on the chip [3]. Despite the advantages in reduced total system footprint, response time and swept volume, a drawback of on-chip actuation approach is that systems can become highly specialized thereby limiting their versatility and possibly reducing fabrication yield.

The presented system distinguishes itself from other integrated systems [3,4] by having more fluidic inputs/outputs, accepting polymeric and silicon/glass based chips, not relying of pneumatics for fluidic control and being compatible with unobstructed, high quality real-time transmission and fluorescence microscopy observation. We demonstrate how the system can be used to switch between different liquid inputs, which is necessary for programming purposes, and that cells can be seeded and cultured on-chip in a microscope for several days.

SYSTEM DESIGN

In the present system we have implemented fluid actuation by miniaturized peristaltic pumps of the type presented in [5] modified for direct attachment to a cell culture chip by integrated ball joint interconnection blocks [6] without any intermediate fluidic components. This significantly reduces swept volume and compliance compared to distantly placed volume displacement pumps. Thus, except from the power supply and electrical controller, every component for driving the microfluidic chip is on the system. It can therefore easily be moved from cell culture labs for cell loading to microscopes for observation just by connecting one single electrical cable through a standard DA-15 connector (Fig. 1).

![Figure 1: a) The complete platform mounted with chip, eight inlet reservoirs and two outlet reservoirs. Miniaturization of pumps and connections allows these and the observation area to fit between life science objectives and a 26 mm working distance (WD) condenser in an inverted microscope. b) Schematic side view showing fit of central parts between 26 mm WD condenser and 2.9 mm WD objective.](image-url)

The platform (Fig. 1), consists of three miniaturized 8-channel peristaltic pumps [5], inserts with space for up to 30 reservoirs and a chip holder allowing the exchangeable chip to be readily snapped on to 32 self-aligning fluidic ball joint...
interconnections [6] by tightening four stainless steel bolts. The exchangeable microfluidic chips are fabricated by direct micromilling in poly(methyl methacrylate) (PMMA) and contain passive microfluidic networks. Depending on the assay requirements the chips can make use of all or a fraction of the inlets and contain single or multiple fluid channel layers for parallel, serial or combinatorial operation all using the same standardized interconnection and actuation platform. All chips are designed with a total of 24 inlets in groups of 8 and a group of 8 outlets and have a microscope slide footprint for ease of use and compatibility with standard life science equipment.

The central element of the pumps containing integrated channels and ball joint interconnections is made of injection molded PDMS using a fabrication scheme similar to the ones previously reported in [5] and [6]. This low-cost element can be exchanged easily between experiments to avoid cross contamination.

Pump control is obtained through a single electrical interconnection to a custom-made controller. A VBA script in Zeiss AxioVision 4.8.1 (Carl Zeiss, Germany) has been developed to simultaneously control the pumps and Zeiss Axio Observer microscope (Carl Zeiss, Germany) for automated perfusion, imaging and analysis.

Cell loading is carried out by pipetting cell suspension into integrated wells at the outlet side. A uniform cell seeding is readily achieved upon reversing the flow. Due to the design of wells and channel system no agitation between pipetting steps is required making it easy to load different cell lines in each well. When sterilized and assembled the system is fully enclosed and can be transferred between equipment without risk of contamination.

EXPERIMENTAL

Without on-chip valves the switching between compounds must be accomplished by start and stop of the low-compliance pumps, which entails a diffusion limited leakage from channel intersections to the downstream culturing chambers. This, however, can be controlled by design to be without physiological significance.

To test the switching capabilities chips containing T-junctions have been fabricated with inlets connected to two pumps. One inlet feeds a fluorescent diluted Streptavidin-Cy3 (S-Cy3) solution (S6402, Sigma-Aldrich, MO, USA) and the other a nonfluorescent phosphate buffered saline (PBS) solution (P5368, Sigma-Aldrich, MO, USA). The switch rate is determined by measuring the average pixel intensity of the fluorescent signal downstream the junction after changing pump settings.

Long-term cell culture directly on a microscope is demonstrated using a PMMA chip with culturing chambers having a cross section of 200 µm by 1500 µm. HeLa Tet-On cells (Clontech 631155, Clontech Laboratories, Inc., CA, USA) have been cultured at an average flow rate of 250 nL/min to test proliferation and viability.

To demonstrate gene expression the line has also been transduced with the fluorescent reporter gene ZsGreen1-DR and cultured under the same flow conditions with added doxycycline at a concentration of 5000 ng/mL. During perfusion the setup is mounted in an inverted microscope (Zeiss Axio Observer) equipped with an incubator to ensure stable temperature and CO2 conditions.

RESULTS

Figure 2 demonstrates how two pumps are used to switch between S-Cy3 and PBS with a downstream diffusion limited leakage. The fluorescent signal is reduced approximately two orders of magnitude within 3 minutes at a flow rate of 250 nL/min in channels with 400x150 µm² cross section.

The result is in fair agreement with time-dependent 2D convection-diffusion finite element (FEM) simulations implemented with a constant inlet velocity in COMSOL Multiphysics 3.4 (COMSOL AB, Sweden). This verifies the system’s switching properties. Switch characteristics can be tailored by changing geometry and flow rate, which has been experimentally verified, and proves the system suitable for cell studies with temporally varied exposure profiles.

Figure 3 shows a long-term time-lapse series of HeLa Tet-On cells cultured an average flow rate of 250 nL/min per chamber. The proliferation and adhesion proves the seeding and perfusion method suitable for the application.

![Figure 2: Switching sequence between PBS and fluorescent S-Cy3 conjugate in a T-junction with channel cross section of 400x150 µm² at a flow rate of 250 nL/min. Arrows indicate flow settings under a) initial condition and b) 8 s, c) 16 s and d) 112 s after switching. Quantification by background corrected, relative pixel intensity over three repetitions and theoretical data based on a FEM simulations is given in e). The box annotation in a) indicates the analysis region.](image)
Figure 3: HeLa Tet-On Advanced Cell Line cultured in a PMMA chamber with a height of 200 mm and 3.2 mL volume perfused with an average flow rate of 250 nL/min. Phase contrast time-lapse images acquired after a) 0 hours, b) 16 hours, c) 32 hours and d) 64 hours of perfusion demonstrate cell adhesion and proliferation.

A gene expression assay is given in Fig 4, which shows a fluorescent time lapse series of transfected HeLa Tet-On cells stimulated with doxycycline over 36 hours initiated after 5 hours of low flow without doxycycline. The signal is seen to increase during the course of the experiment thus demonstrating a well functioning gene expression.

Figure 4: HeLa Tet-On Advanced Cell Line showing overall increased expression of a transfected fluorescent reporter gene ZsGreen1-DR during perfusion at a rate of 250 nL/min with medium containing doxycycline. Images a)-d) show fluorescent micrographs of the cells at 0, 8, 16 and 24 hours after stimulation start, respectively. Image e) shows the fluorescent signal over 36 hours measured as average pixel intensity in a 3 mm² area normalized to the first frame.

CONCLUSION

We have presented a complete, self-contained platform for programmable long-term cell culture with microscope access. Using the platform we have demonstrated that two different fluids can be exchanged with two orders of magnitude within few minutes at relevant culturing flow rates and that cells can be cultured and stimulated over days with the system mounted in a microscope.

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REFERENCES


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