Dynamic cell culture: a microfluidic function generator for live cell microscopy

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Received 6th May 2008, Accepted 18th August 2008
First published as an Advance Article on the web 20th October 2008
DOI: 10.1039/b807682k

We present a microfluidic system for time-lapsed, live cell microscopy with the ability to control solution exchange *via* a dynamic flow controller. The application specific microfluidic plates are designed to maintain adherent and non-adherent cell types for multiple days with continuous medium perfusion. Upstream channels with flow controlled *via* custom software allow the delivery of unique exposure profiles to the cultured cells, such as square waves, step functions, ramps, *etc*.

Introduction

The utilization of microfluidic devices for cell culture applications holds tremendous promise for the future of biology.[1] This approach enables the engineering of microenvironments at the cellular size scale[2] with miniaturization and automation of complex protocols.[3] In order to mimic the physiological environment of cells in tissues, a number of groups have successfully demonstrated the ability to create continuous flow arrays for *in vitro* microfluidic culture.[4–7] More recently, this capability was enhanced with systems capable of exposing cells to changing flow environments, enabling a type of "signal-response" experiment not previously possible.[8,9] Increased use of dynamic cell culture systems may provide novel insights into cellular processes that were not accessible with conventional static culture methods.

In this work, we describe the design and engineering of a robust microfluidic cell culture device with a straightforward interface for dynamic medium exposure. This concept is based on the use of disposable, application specific microfluidic plates and a universal flow control interface. The microfluidic plates house all sample solutions, media, and cells, and is formatted with a 96-well plate footprint, with 10 pipette accessible inlet/outlet well positions. Flows through the microfluidic device are actuated *via* precisely regulated pneumatic pressure routed to each of the 10 well positions.[9] The microfluidic cell culture chamber is based on previous work, with the key feature being a perfusion barrier to control nutrient transport and cell localization.[10] This design maintains the cultured cells in a specified imaging region with exposure to continuous perfusion of media solutions. A glass coverslide bottom and transparent optical path facilitate high magnification imaging on an inverted microscope.

Experimental

The components of the dynamic culture system are depicted in Fig. 1. The microfluidic cell culture chamber is 1 × 3 × 0.05 mm in size (150 nL), with channels for cell introduction, solution exposure, and flow outlet. A perfusion barrier consisting of a grid of 5 × 4 um cross section channels prevents cells from moving into the flow channels. In order to prevent backflows between the multiple solution inlet channels, a region of high fluidic resistance is placed upstream of the culture chamber. The high resistance is

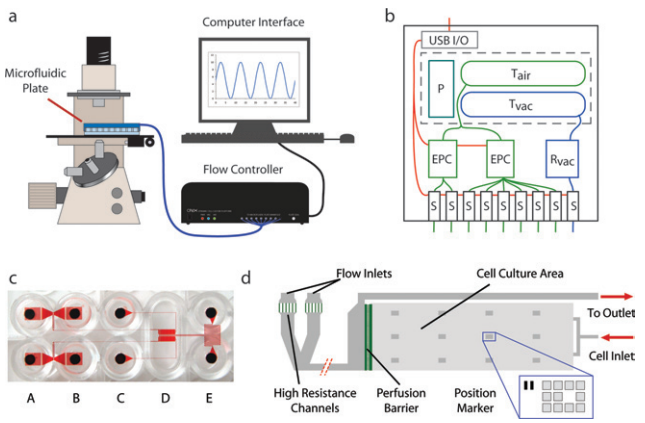

Fig. 1 Microfluidic dynamic cell culture system. (a) The components of the system include a computer software interface, a pneumatic flow controller, and a transparent manifold sealed to the microfluidic plate placed on a standard inverted microscope. (b) Schematic of the flow controller hardware, depicting the digital control card (USB I/O), internal pressure/vacuum generator (diaphragm pump, P, compressed air tank, T_{air}, vacuum tank, T_{vac}), two electronic pressure controllers (EPC), vacuum regulator (R_{vac}), and nine 3-way solenoid valves (S). The nine outlet lines are routed to the microfluidic plate *via* the manifold to allow pressure driven flow through the microfluidic inlet wells. (c) The microfluidic plate layout uses multiple open wells that conform to standard 96-well size and spacing. The dynamic mixing design is depicted, where 2 inlet solutions (columns A and B) are mixed upstream prior to exposure to 2 separate cell culture chambers (column D). The wells in the top row are routed to chamber 1, and the bottom set of wells are routed to chamber 2. Cells are introduced from column C, and E is the flow outlet. (d) Expanded view of the microfluidic cell culture region showing the flow inlet lines, cell inlet, and flow outlet paths. The culture area is a 3 × 1 mm rectangle with embossed position markers to facilitate microscopy. A 4 μm tall perfusion barrier localizes cells to the desired region and promotes uniform flow exposure. Upstream high resistance regions prevent backflows between the inlet wells, as well as normalizing any downstream pressure fluctuations.

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created by a set of narrow cross section channels (5 × 2 μm) in each of the flow inlet channels, resulting in a flow rate ratio of 200 : 1 between flow downstream to the cells and outlet *vs.* flow back upstream to the solution channel.

The microfluidics are placed under the well positions of a customized 96-well plate. This consists of 5 columns (A–E) and 2 rows. The cell culture chamber is underneath an "imaging window" in column D. The other wells are designated for solution inlets, cell inlets, or flow outlets, depending on the application specific design. The plate seals to a transparent manifold, which provides an interface to the pressure flow controller. This configuration eliminates tubing connections, and confines sample contact to the microfluidic plates, facilitating setup and cleanup. The pressure based flow controller consists of an internal pressure/vacuum pump, electronic pressure controllers, and a set of solenoid valves, commanded *via* a computer with custom software. Flow profiles were programmed into the software and executed during imaging. This flow control system is commercially available from CellASIC Corp (San Leandro, CA, USA).

Cell culture and time lapse microscopy were carried out according to the following steps. First, 10 μl of HeLa cell suspension (10^6 cells ml^{-1}) was pipetted into the cell loading well (E). Cells were introduced at a pressure of 0.4 psi until a suitable number of cells filled the culture chamber. The cells were perfused at 50 μl day^{-1} with CO_2 Independent Medium (Invitrogen) with 10% FBS in a heated 37 °C microscope enclosure. Time-lapsed imaging showed cell attachment and growth over 24 hours (Fig. 2). The cells survived for 7 days on the microscope stage under continuous perfusion (at ~20 μl day^{-1}) without medium replacement. The continuous perfusion as well as the sealed manifold enabled long term culture during imaging without the need for a humidified chamber or CO_2 gas control.

Function generation of exposure solutions was programmed into LabView (National Instruments) by sequencing flows and

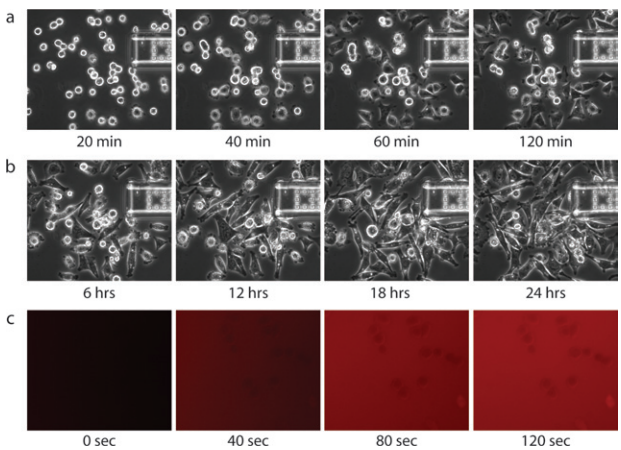

Fig. 2 Cell culture operation. (a) HeLa cells were loaded into the microfluidic culture chamber and observed in a temperature controlled microscope chamber with continuous perfusion of CO_2 Independent Medium (Invitrogen). After 2 hours, the cells attached and spread on the glass chamber floor. (b) Over 24 hours on the microscope stage, high cell viability and growth was observed. (c) Images taken as the fluorescent dye was introduced to the cells showed the uniform profile of solution exchange.

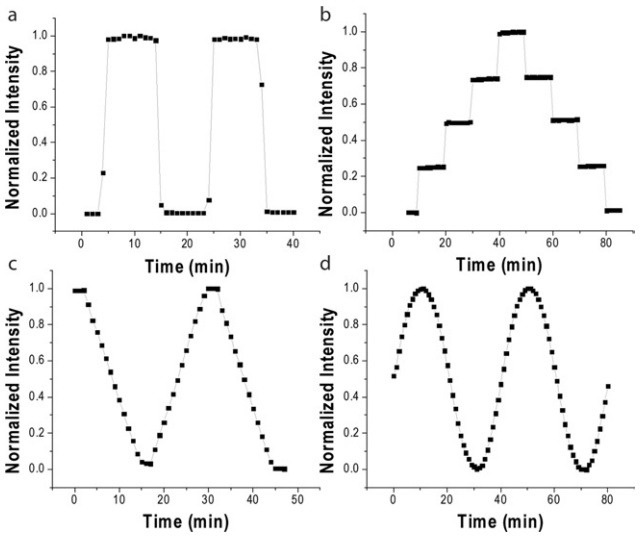

Fig. 3 Dynamic exposure functions. The system was used to generate dynamic exposure profiles to cultured cells measured *via* fluorescent dye intensity. This demonstrated the ability to create: (a) a square wave, (b) an ascending and descending staircase, (c) a ramp, and (d) a sinusoidal wave.

pressures. We demonstrated three basic function profiles: binary switching, multiple switching, and dynamic mixing (Fig. 3). These functions were performed on three microfluidic devices, each with a slightly modified inlet channel design. The binary switching microfluidic device contained 2 flow inlet channels, the multiple switching design had 5 separate upstream inlet channels, and the dynamic mixing design utilized continuous laminar flow mixing between two upstream channels. The time scale of exposures was on the order of minutes to hours, which is typical of cellular processes such as gene expression, motility, and mitosis. The liquid front traveled through the culture chamber in a laminar profile ($Re < 0.03$), traversing the 3 mm length of the cell culture chamber in roughly 1 minute. Data collection was performed by time-lapsed fluorescence imaging of Texas Red conjugated dextran (MW 10K) at the front 1/3 of the culture chamber on an inverted microscope (Olympus IX71). Intensity was normalized by linear extrapolation to the maximum and minimum values for each run. Profiles were created by specifying the pressure output from each of 2 electronic regulators and the open/close state of 8 solenoid valves.

Binary Switching. This function created a simple "on/off" switch between two exposure solutions. The resulting profile was a "binary" square wave, where the cell was exposed to either 0 or 1 (typically an experiment will use a buffer solution "0" and an exposure solution "1"). In this profile, the exposure was switched between phosphate buffered saline (PBS) solution and Texas Red conjugated dextran with an on/off time of 10 minutes at an applied pressure of 8 psi.

Multiple Switching. An extension of the binary switch profile, this configuration allowed switching between 5 input solutions. Here, we used discrete concentrations (0, 0.25, 0.50, 0.75, 1.0) to create a step function. Solution exposures were changed every 10 minutes at an applied pressure of 8 psi. This device can also be used to expose cells to completely unrelated

solutions, such as different drugs, lysis solution, fixatives, or immunostaining.

Dynamic Mixing. This design used two input solutions to create continuous mixtures by controlling the relative flow rates of each component. As the two solutions flow down a 50 μm wide and 20 mm long channel, diffusive mixing created a homogenous solution well before reaching the cells (typically 20–30 seconds). The most common application of this function is to generate changing concentration exposures over time. Here we demonstrated the ability to produce a ramp function and a sinusoidal function. This device can also be used to create the staircase function shown for the multiple switching design. The two boundary conditions (PBS and Texas Red conjugated dextran) were dispensed into the two separate mixing inlet wells. The total pressure was kept fixed at 10 psi. To produce the ramp and sinusoidal function, incremental steps of the mixing ratio was performed every 20 seconds.

Discussion

The goal of this work was to develop a flexible and robust microfluidic system for dynamic medium exchange during live cell microscopy. By sequencing pressure driven flows from multiple inlets routed to a single microfluidic culture chamber, we were able to demonstrate exposure functions including: binary (on/off) switching, ascending and descending stair steps, ramps, and sinusoidal waves. While live cell microscopy has shown tremendous progress in recent years, most experiments are still performed in static culture dishes. The options available for dynamic experiments are limited, and typically rely on a cumbersome "flow cell" apparatus or laboratory-specific microfluidics driven with syringe pumps. Compared to other microfluidic control systems,[7,11,12] the key advantage of the current work is to provide reliable and easy to operate devices for the non-specialist. This approach does not incorporate complex features such as on-chip valves, multi-phase flows, micro-electrodes, bio-materials, or nanostructures. We believe that providing life-science researchers with a well designed tool for microfluidic cell analysis will open the door for future adoption of more sophisticated lab-on-a-chip technologies.

The flexibility of the system emphasizes our "application specific" design concept, such that additional microfluidic plates can be generated depending on the intended experiment. Here, we demonstrated three application specific plate designs (binary switching, multiple switching, and dynamic mixing), but it is straightforward to implement any number of new features, such as spatial gradients, recirculating flows, multiple cell co-cultures, cell-specific culture chambers, multi-phase flows, *etc.* In addition, the ease of inputting precise exposure profiles *via* the software interface enables a limitless set of experiment conditions that can be explored for live cell microscopy. This ability to observe real-time cellular responses to dynamic environmental cues will provide unique insight into pathway interactions, intracellular signal processing, and the biochemical "computing" performed in live cells.

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