Automated cell culture in high density tubeless microfluidic device arrays†

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Microfluidics is poised to have an impact on life sciences research. However, current microfluidic methods are not compatible with existing laboratory liquid dispensing and detection infrastructure. This incompatibility is a barrier to adoption of microfluidic systems and calls for improved approaches that will enhance performance and promote acceptance of microfluidic systems in the life sciences. Ease of use, standardized interfaces and automation remain critical challenges. We present a platform based on surface tension effects, where the difference in pressure inside drops of unequal volume drives flow in passive structures. We show integration with existing laboratory infrastructure, microfluidic operations such as pumping, routing and compartmentalization without discrete micro-components as well as cell patterning in both monolayer and three-dimensional cell culture.

Introduction

In spite of numerous demonstrations of biochemical and physical control made possible by microfluidic systems, experiments employing these tools represent a very small fraction of those reported in the life science literature to date. The goal of the work described in this paper was to develop devices that life scientists may use without the direct help of microfluidics engineers.

The use of microfluidic systems often requires expertise that is not part of life science training. Consistently, most biological studies employing microfluidics have had microfluidics engineers directly involved. Some of the expertise is related to fluid flow control. Off-chip syringe pumps are relatively simple to set up, but require sensitive handling of tubing to avoid disturbing flow. On-chip peristaltic pumps driven by off-chip pneumatics rely on significant external pneumatic hardware and control software. Electro-kinetic flow necessitates management of bubble formation, heating and buffer conditions. Furthermore, all of these methods require physical connections to the chip during pumping which make it cumbersome to use the systems with traditional cell culture equipment, such as incubators and microscopes. There is an unmet need for easy-to-use microfluidic systems.

Although a variety of interfaces have been developed for microfluidic systems, no standards yet exist for interfacing with the macro-world. This is in stark contrast with life science research, where pipettes are the most pervasive liquid handling tool and micro-titer plate standards (ANSI/SBS 1–4, 2004) are universally adopted. Over the past 15 years micro-titer plates have been scaled to high densities. Alongside high density micro-titer plates, pipettors and other liquid handling instrumentation have continually been improved and now offer parallelism (e.g. 96 or 384 tips), high accuracy metering and positioning of droplets ranging from picoliters up to hundreds of microliters. Microfluidics would benefit from similar interface and automation standards. Here we demonstrate a microfluidic platform that leverages and adopts these same standards.

From an experimental perspective, micro-titer plates offer life scientists an easy way to study several conditions in parallel in separate compartments. Compartmentalization is also useful for microfluidic cell culture devices. This need has been addressed by using pneumatic valves to multiplex inputs. However, pneumatically isolated chambers provide only limited opportunity for microfluidic manipulation (e.g. controlled flow and cell patterning). Furthermore gels are difficult to manage in the channel networks needed for multiplexing. Three-dimensional culture is gaining ground in basic research and thus it is important to identify methods that combine compartmentalization, parallelism and ability to use gels. Various methods have been employed to produce three-dimensional cell culture in microfluidic systems including laminar flow, photopatterning, microarray spotter, and molded gel compartments. Although the last two methods allow tests to be performed in parallel, those tests will all be performed in the same fluid medium limiting the range of experimental conditions that can be attained (i.e. lack of compartmentalization).

Additionally, pneumatic multiplexing requires an elastic construction material. Silicone rubber (poly(dimethylsiloxane), PDMS) is the most commonly used material in microfluidics research. While PDMS is an excellent prototyping material, it is plagued by bulk absorption of small hydrophobic molecules. This, in turn affects the outcome of many cell experiments due to absorption of bio-active small molecules, such as drugs. While pneumatically controlled integrated microfluidic array systems

†Electronic supplementary information (ESI) available: Videos showing passive pumping and interfacing by a 96-tip robotic pipetter as well as additional results for compartment division and joining. See DOI: 10.1039/b715375a
have had great success in industrial biochemical applications, their use in cell biology must be applied carefully due to hydrophobic molecule absorption.

Several of the issues described above can be addressed by using droplet-based passive pumping. Through analytical modelling and experimentation we have shown that passive pumping occurs in two phases characterized by constant wetted area and constant contact angle, respectively. The two-phase pumping leads to a relatively uniform flow velocity which is accurately predicted by the analytical model. According to the model, a 1.5 μl input drop dispensed to a channel that is 0.5 mm wide, 4.5 mm long and has height in the range of 25–250 μm, will produce a time averaged flow rate in the range of 34.6 nl/s to 16.6 nl/s. We have further shown that by choosing an appropriate input volume relative to the channel volume, any given fraction of fluid replacement can be achieved. This may require multiple additions and aspiration steps, depending on the volume of the channel and the amount of fluid replacement desired.

Here we present a simple microfluidic platform based on passive pumping. The only skill needed to operate the platform is the use of a pipette to touch off drops on a surface. The microfluidic devices require no pumps and are built in plastic tissue-culture trays with lids that are in all aspects compatible with traditional cell culture equipment. Importantly, all fluid manipulation can be achieved using a variety of materials. Passive pumping with the standard micro-titer plate footprint (SBS/ANSI standard) is demonstrated. We also show that automated and manual pipetting tools, including automation control software can be used off-the-shelf to perform cell-based assays microfluidic devices via passive pumping. Importantly we demonstrate a high degree of parallelization (96–192 channels per array) while retaining basic microfluidic operations including routing, compartmentalization and laminar flow. Applications to patterning of multiple monolayer cell colonies and three-dimensional cell compartments and co-culture are demonstrated.

Methods

Device fabrication

An array of microchannels was cast in poly(dimethylsiloxane) (PDMS, Dow Corning) from masters prepared by photolithography using SU-8 photoresist (Microchem, USA) on 6-inch silicon wafers. The array was mounted in a tissue-culture treated plate (Omnitray, Nunc). The array, dubbed a micro-conduit array (MCA), has 192 straight channels with rounded ends, 5.5 × 0.75 × 0.25 mm L × W × H and access ports positioned according to microtiter plate standards as shown in figure 2(a-b). The distance between ports was 4.5 mm center-to-center. Multi-input devices for routing and laminar flow experiments were made using similar methods with channel heights 0.250 mm (for width and length information see scalebars in figures 3 and 5). Laminar flow structures were constructed such that the resistance from each input to the main channel was equal. To ensure accurate registration between automated liquid handlers and the MCA ports two approaches were used. Shrinkage of PDMS can be pre-emptively corrected in master mold. Alternatively, the location of access ports can be modified appropriately in the liquid handling automation control software.

PDMS devices were autoclaved and subsequently combined with sterile PS tissue culture plates via reversible bonding to produce sterile devices for cell culture.

Device operation

The channels of the MCAs are hydrophobic and thus need to be filled prior to use. The MCA was covered with either phosphate buffered saline (PBS, Gibco) or Dulbecco’s modified Eagle Medium (DMEM, Gibco) and placed under vacuum in order to fill the channels with DMEM (similar to method described in reference 24). Each channel was thereby filled with PBS or DMEM, such that the fluid was flush with the top surface of the device.

Hand pipettes (Eppendorf, Gilson, Brand) were used to achieve passive pumping. Volumes ranging 2–30 μl were dispensed at the output ports and volumes ranging 0.3–15 μl were dispensed at the inputs, the latter volume always being smaller than the first. For volumes smaller than 8 μl the fluid was dispensed to a drop at the tip of the pipette and touched off on the surface. For volumes greater than 8 μl (and low surface tension fluids) drops disengage from the tip due to gravity and thus do not need to be touched off.

Automated pipettors (223 Sample changer, Gilson USA; Biomek FX, Beckman Coulter, USA) were programmed to deliver drops the same way as described above. Pipettor control software is designed for use with microtiter plates. The MCA access ports are arranged according to microtiter plate standards (ANSI/SBS 1–4–2004), and the microtiter well maps in the software were used directly to prescribe the fluid source (typically a 96 well microtiter plate), what quantity to dispense, at which access ports and in what order.

Fluid flow patterns were visualized using three different methods: dyed water (McCormick food dye) as shown in figures 1(e–h), 2(a–d), and 3; fluorescent dye solution (Fluorescein isothiocyanate, Sigma) as shown in figure 4(d); and using fluorescent bead suspensions (Fluospheres Red and Crimson, Molecular Probes - Invitrogen) as shown in figures 4(b-c) and 5(a).

To achieve laminar flow an 8-channel pipette was used (BrandTech, Germany) to simultaneously dispense 2 μl droplets (see figure 5(d-e) or 8 (see figure 5(a-b) ) drops of different solutions.

A liquid bridge was formed between microchannels as follows. Two adjacent channels were prepared with 2 μl output drops and each seeded with 1 μl of different color fluorescent bead suspension. A 1 μl drop of buffer was added to the output of the second channel to ensure that port superceded the liquid bridge as an output. Three evenly spaced 0.5 μl drops of cell culture medium (protein content helps wetting) were placed between the two access ports (2.25 mm center to center). The drops spontaneously coalesced to form a liquid bridge. Five 1 μl drops of fluorescent dye solution were dispensed in sequence at the input port of the first channel and flowed through the first channel, the liquid bridge, and the second channel to reach the output of the second channel.

Cell culture

All manipulations of cells including seeding were performed in a laminar flow biohood and cell culture was conducted in a traditional cell culture incubator.

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Pre-adipocytes (3T3-L1, ATCC) were harvested from a cell culture flask and suspended in cell culture medium at 2 × 10^6 cells/ml and placed in a 96 well plate (Nunc 249662 conical bottom), along with cell culture medium (Dulbecco’s Modified Eagle’s Medium (DMEM), Gibco) supplemented with 10% fetal bovine serum (FBS, Biomedia) and 10 mg/ml Insulin (Sigma). The cells were washed with phosphate buffered saline (PBS, Gibco), removed from the flask using 0.05% Trypsin (Gibco) and placed in culture medium. The cells were centrifuged (1000 rpm for 5 minutes) and resuspended in DMEM + 10% FBS. NMuMG and GFP-NMuMG cell suspensions were prepared at 2 × 10^6 cells/ml in a 96 well plate (Nunc 249662 conical bottom) along with cell culture media. The cells were seeded in an eight input laminar flow plate (Nunc 249662 conical bottom) along with cell culture medium (Dulbecco’s Modified Eagle’s Medium (DMEM), Gibco) supplemented with 10% fetal bovine serum, FBS) for seeding in an MCA. Using an automated pipettor (223 Sample changer, Gilson, USA), 6 μl of cell culture media was transferred to the channel outputs, followed by transferring 3 μl media to all channel inputs twice and aspirating 6 μl from all channel outputs. Last, 3 μl of cell suspension was transferred to 130 channels, 3 μl cell culture medium was transferred to 10 of the remaining inputs (intended as control for fluorescence background), and 3 μl was aspirated from all outputs. After seeding, the MCA was placed in a large bioassay dish (Nunc) containing filter-sterilized reagent grade water, and that dish placed in a cell culture incubator at 37°C and 5% CO₂. The large surface area of water in the bioassay dish helps keep the environment inside and around the MCA saturated with water vapor and thus reduce evaporation. The cell culture media was changed every 24 hours by transferring 3 μl to all inputs, aspirating 9 μl from all outputs (i.e. almost all the volume), followed by two cycles of adding 3 μl culture media to all inputs.

Mouse mammary epithelial cells (NMuMG from ATCC and GFP transfected NMuMG cells generously provided by Professor Hoffmann) were cultured in a cell culture flask in Dulbecco’s Modified Eagle’s Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Biomedia) and 10 mg/ml Insulin (Sigma). The cells were washed with phosphate buffered saline (PBS, Gibco), removed from the flask using 0.05% Trypsin (Gibco) and placed in culture medium. The cells were centrifuged (1000 rpm for 5 minutes) and resuspended in DMEM + 10% FBS. NMuMG and GFP-NMuMG cell suspensions were prepared at 2 × 10^6 cells/ml in a 96 well plate (Nunc 249662 conical bottom) along with cell culture media. The cells were seeded in an eight input laminar flow MCA (one input not used) by simultaneously transferring seven fluids from the 96-well plate to the MCA using an 8-channel pipettor (BrandTech Transferpette, Germany). The seven fluids were: GFP-NMuMG cell suspension, media, media, NMuMG cell suspension, media, media, GFP-NMuMG cell suspension.

Fibroblasts (Hs578Bst) and epithelial cells (HMT-3522 S1) were stained respectively with CellTracker Red and CellTracker Green (Molecular Probes - Invitrogen). Cells were then loaded by passive pumping into microchannel devices. Simultaneous addition of 2 μl of cell-collagen (Rat-tail collagen I, BD Biosciences) droplets led to passive pumping and laminar flow, resulting in the creation of two discrete cellular compartments after gelation. By simultaneously pumping MCF10A cells embedded in 3D lrECM (Matrigel, Growth Factor Reduced/Phenol Red Free, BD Biosciences) alongside non-gelling viscosity matched solution (PEG8000, CarboWax, Fisher), an open aqueous channel was created adjacent to the 3D cellular compartment. Following gelation, the viscous solution was passively pumped out of the channel.

**Results**

In a tube-less microfluidic system, holes through the top surface serve as access ports. Passive pumping is employed to deliver reagents and produce flow as shown in figure 1.

The pumping pressure is produced by the surface tension in the curved surface of liquid droplets placed on the access ports as shown in figure 2(a-d). We found passive pumping to work robustly with significantly smaller output drops than previously described (output drops were referred to as the reservoir in reference 21). Output drops down to 2 μl were used with correspondingly smaller input drops. This allowed for the arrangement of channels in closely spaced arrays as shown in figure 2. With arrays formatted with port-to-port spacing equal to the well-to-well spacing in microtiter plates we successfully used handheld pipettes (figure 2(a-d), and up to 96-tip automated liquid handling robots (figure 2e) to deliver fluids to MCA devices (see videos in supplementary information). The liquid handling robot control software was used off-the-shelf and titer plate well maps were used to designate microchannel access ports as inputs and outputs for flow. Using the well maps, a sequence of aspirate and dispense commands was programmed to transfer fluids from a micro-titer plate reservoir to the microfluidic array. The program was executed to replace the buffer in the microfluidic channels with cell culture media.

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**Fig. 1** Passive pumping. a) Empty microfluidic channel. b) Channel filled with a fluid using a pipette and a drop (output drop) being formed on the right port. c) Introduction of a smaller volume of a second fluid (input drop) at the left port and pumping starting to occur. d) The volume of the input drop is transferred into the channel, displacing the first fluid by the second fluid until input and output drops reach equilibrium at equal radii of curvature. e) A 1 μl input drop is dispensed at the left access port of a water-filled channel that has a 6 μl drop at the output port. f-h) Flow starts as soon as the drop makes contact with the port and continues until equilibrium is reached.
and subsequently a cell suspension. Cells were cultured over 5 days with media changes every 24 hours. An MCA immediately after cell seeding is shown in figure 2(e) and a phase contrast photomicrograph showing cells (3T3-L1 pre-adipocytes) on day 5 in culture are shown in figure 2(e) inset. When using 1-, 8- and 96-tip liquid handling robots the dispense height and dispense rate were the most important parameters. Once optimized, drops down to 2 μl could be touched off at a greater than 95% success rate across arrays repeatably between devices (lower volumes should be feasible with more precise pipetting systems). Alignment of the microchannel arrays in the tray was also important for successful operation as well as accounting for shrinkage in the PDMS casting process.

For passive pumping the air-liquid interface with the greatest radius of curvature (lowest pressure) will become the output and flow will occur in a point-to-point fashion from the inputs to that output. The application of this principle to fluid routing is shown in figure 3. After a drop is added at one access port the air-liquid interfaces at all access ports seek a common equilibrium.

Fig. 2 Device geometry and standard interfacing. a) Geometry of a microchannel array with access ports arranged according to micro-titer plate standards (dimensions in mm). b) Top view of a single channel showing output and input ports. c) Side view of a single channel showing device construction. d) An MCA interfaced by a 96-tip liquid handling instrument (Biomek FX, Beckman Coulter, USA). e) An array of 192 microfluidic channels each with two access ports positioned according to micro-titer plate standards. Inset: Pre-adipocytes (3T3-L1) on day 5 in culture (scalebar: 1 mm).

Fig. 3 Routing. a) Four-way junction microfluidic structure (channel cross-section: 1 mm × 0.25 mm, W × H). b) A large drop was dispensed at the top access port of a four-way junction and three drops, red (b), blue (c) and yellow (d) were dispensed at the left, right and bottom access ports, respectively and flowed point-to-point to the output (scalebars: 1 mm).

Fig. 4 Selective joining of compartments. a) Two adjacent microchannels, each with two access ports. b) Two chambers were loaded with fluorescent microbeads (scalebars: 1 mm). c) A small drop of wetting fluid was dispensed between the channels and formed a continuous liquid bridge connecting the two channels. d) Green fluorescent dye was dispensed at the left input of the left channel and flowed to the right output of the right channel. e) A schematic showing the liquid bridge.
The curvature of the output drop dictates the curvature of the air-liquid interface pinned at the rim of each remaining access port. For small volume additions the curvature of the output drop does not change significantly as it receives the newly added volume. Neutral ports (those without any droplet) are unaffected by the pumping drop. As a result no fluid mass is transferred into the branches leading to ports other than the output port, except that due to the flow profile at the intersection.

Each element of a tube-less array is a separate compartment. The ability to selectively connect two compartments is advantageous when entities are bound in space, such as when culturing adherent cells. By wetting the surface between two access ports a stable liquid bridge is formed that permits diffusion (of particles) or convection (bulk fluid flow) between select compartments as shown in figure 4, thus joining the two compartments. We have also demonstrated the use of hydrophilic/hydrophobic patterning to confine the liquid bridge to a predetermined area and make the bridge bistable such that compartment can be disconnected at a later time (see supplementary information).

Multiple stream laminar flow was achieved via passive pumping as shown in figure 5a). Using hand pipettors two stream flow worked very repeatably, but four and eight stream flows require more practice to achieve simultaneous touch-off of all inputs without wetting excess area around inputs. Other factors affecting stream width and duration include the dispensed volume accuracy, the timing of dispense and the wetted area. Precise, simultaneous touch-off may easily be achieved with automated pipettors. When using 20 μl output and 8 μl input drops eight-stream laminar flow lasted approximately 2 minutes. For those conditions the average flow rate was 67 nl/sec. Flow duration ranging from less than 1 second to several hours are easily achieved via appropriate choice of relative droplet sizes and channel resistances.

Multi-stream laminar flow was employed for cell patterning as shown in figure 5(b-d). Fig. 5(b) shows cells (NMuMG and GFP-NMuMG) patterned in monolayer culture (on tissue-culture treated polystyrene) via settling from laminar streams. Three dimensional co-culture was also achieved. Fibroblasts (Hs578Bst stained red) and epithelial cells (HMT-3522 S1 stained green) suspended in fluid collagen were loaded in a two-input microchannel (2 μl at each input) shown in figure 5(c) The collagen cell suspension was kept on ice and the microfluidic channel array at room temperature. Upon seeding the collagen flowed into the channels and gelled as it warmed up yielding two discrete cellular compartments within a contiguous three-dimensional gel as shown in figure 5(d) In a separate device a suspension of MCF10A cells in fluid IrECM were pumped alongside a non-gelling viscosity matched solution. Upon gelling of the IrECM, an open aqueous channel was created adjacent to the 3D cellular compartment. Following gelation, the viscous solution was passively pumped out of the channel and replaced by cell culture media. Fig. 5(e) shows the cells in a gel compartment adjacent to an open aqueous channel at 4 days post plating.

Discussion

We have developed new methods based on passive pumping that can be realized in rigid materials and achieve basic microfluidic functions without external or integrated structures for pumping and valving. Experimental cell biology often requires compartmentalization. Direct access to individual elements in an array via holes in the surface reduces the need for multiplexing and
segregation of compartments by valves in microfluidic device arrays.

The remaining functions of valves are routing of flow and selective division or joining of specific compartments. Routing (figure 3) was demonstrated as well as selective division (see supplementary information) and joining of compartments (figure 4) simply by adding and removing drops on the surface of the microfluidic device. We further showed laminar flow via passive pumping (figure 5). An arbitrary number of parallel streams may be produced to achieve microscale control (up to 8 were demonstrated). By using passive pumping to produce laminar flow, tube compliance and inherent variations in mechanical pumps are avoided. Additionally, passive pumping was applied to the laminar flow patterning of three-dimensional cell compartments and aqueous channels adjacent to the gel compartments. Compared to previous approaches to laminar flow patterning of 3D gels, passive pumping greatly simplifies the method of operation and opens the door to automation using existing hardware. The integration of aqueous channels with gels have been shown to facilitate media changes, treatments and staining by reducing the path length for diffusion across the compartment.

The absence of tubes makes MCA devices truly low volume with minimal dead space rendering the system particularly attractive for studies involving rare cells such as adult stem cells and primary cell sub-populations from animals or human biopsies. The reduction in cell number may be 10- to 100-fold per datum compared to traditional life science methods. Importantly, the MCAs enable miniaturization of population-based cell assays in automation-compatible array format without custom instrumentation or long setup times.

The tube-less approach allows robotic instruments to operate on multiple microfluidic devices in series, leveraging microtiter plate methodology to transfer stacks of devices from one station to the next (e.g. barcode labeling, sample pre-treatment, microfluidic assay, incubation, readout). This conveyor belt-type processing is fundamentally different from previously described methods to increase microfluidic system throughput based on gradients, laminar flow interface control or valving. The ability to take advantage of microfluidic phenomena is limited in high density valve arrays. While gradients and laminar flow can allow several distinct tests, constant flow is required to prevent cross-talk between cell populations. Continuous flow dissipates secreted factors and thus prevents signaling and media conditioning. For continuous flow, each fluid requires a designated input tube and thus complexity and setup time scale with the desired number of conditions. As with traditional well plates, MCAs achieve independent conditions across an array such that the number of chemically distinct tests equals the number of microfluidic units. The software complexity scales with the number of different conditions while devices remain simple and setup time short. Microfluidic phenomena can be employed in experiments that are are not limited to a single entity or a titration of a single molecule. Any combination of entities and molecules can be tested and software-defined channel use leads to flexibility in experimental design.

Compatibility with pipettors, both manual and automatic, makes tube-less microfluidic systems immediately compatible with conventional sample preparation and readout. Present off-the shelf liquid handling equipment is designed for the integration of sample pretreatment and readout. Scalability and compatibility with full automation is especially important for microfluidic applications in high throughput screening (HTS), where millions of specific chemical compounds may need to be tested in a short period of time. Arrays of higher density than those shown in figure 2(e-g) are also possible. We have produced arrays of up to 768 micro-chambers in the same 128 × 86 mm footprint (see supplementary information).

A broad range of flow environments can be created using passive pumping. The cell culture experiments described above were all conducted without flow. This is important, since data suggest that no flow cell culture may facilitate in vitro recreation of the biochemical micro-environment in which cells exist in vivo. However, it is necessary to replenish nutrients and remove waste products in long-term experiments. This is often achieved via constant perfusion of cell culture media. It is possible to realize perfusion culture using passive pumping by adding a series of drops at an input, each time introducing a new drop before the volume at the input falls below a given level. Slow flow may also enable media change to be carried out in suspension cell culture without appreciable loss of cells. The lowest flow rates achievable with passive pumping generate negligible shear stress on adherent cells, but at the opposite end of the range passive pumping may allow tunable shear stress for cellular biomechanics studies.

Gas exchange and removal of waste products is important for cell culture devices. Given the oxygen consumption of common cell lines, simple diffusion models indicate that access ports at 4.5 mm spacing that are open to a regulated environment (i.e. incubator) provide sufficient transport to maintain appropriate gas concentration.

Evaporation is an important consideration for all life science tools employing volumes below 10 μl. Consistent with the theme of integration with conventional life science approaches we employed the most commonly used method of evaporation control, which is to place devices in a closed humid environment. Through computational and experimental work we have shown that by placing a lid on MCA devices (or trays of comparable geometry), the enclosed volume becomes saturated with water vapor rapidly and with minimal loss of volume. The local humidity at equilibrium depends on the outside humidity. The local humidity can thus be increased by placing the lidded tray inside a second enclosed, vented container with water covering the base inside a humidified cell culture incubator. The results from our evaporation studies are consistent with our experience that cells can be cultured in MCA devices for over two weeks with a media change interval of 24 hours. Media changes and other fluid manipulations are generally carried out outside a humidified environment. It is important to minimize the duration of such steps. The most dramatic conditions exist in laminar flow bio-hoods. In our experience, for typical MCA access port geometries (1–2.5 mm diameter) and assay volumes (2–10 μl) a time window of at least 6 minutes exists even under laminar flow convective conditions. This allows ample time for robotic fluid handling since 96-tip robotic pipettors can load an array of 192 channels with media and cells in less than 3 minutes. For manual operation, the number of datapoints may be chosen appropriately and the remaining channels filled with fluid (e.g. phosphate buffered saline) to help humidify the tray.
once lidded. The duration of non-humidified operation can be extended dramatically by working without convection (if sterility requirements are less stringent) or by slowing evaporation via cooling.

In the short term micro-titer plate compatible microfluidic systems may improve accessibility by the life science community. In the longer term, specialized liquid handling and readout instruments may evolve together with tube-less systems taking further advantage of passive pumping-driven microfluidic phenomena in both fluid control and analysis. One possibility is the use of passive pumping-based decision making and timing to create autonomous cell-based assays where the appropriate placement of droplets preprograms the assay steps such that they are performed autonomously without further operator or instrument input.

Conclusions

We have merged surface tension driven passive pumping with traditional fluid handling tools to create an easy to use microfluidic platform that expands the capabilities of cell culture based inquiry. This is a simple approach to compartmentalized microfluidic cell culture arrays that is appropriate for low throughput manual operation (hand pipette) as well as high throughput lab automation. In contrast to previous parallelization approaches, tube-less microfluidics provides compartmentalization without sacrificing microfluidic manipulation, and without the need for elastomers that absorb hydrophobic compounds. Tube-less microfluidic systems are scalable to any number of culture conditions and cell types desired. Passive pumping provides the important functions of routing, division and joining of separate compartments, laminar flow, as well as cell patterning in monolayer and three dimensional culture. While the discrete access architecture presented here may seem a step backwards, it allows arbitrary plate layouts in generic devices, in contrast with many existing addressing schemes. The tube-less approach maintains device simplicity by transferring operational complexity from the device to generic liquid handling control software. The combination of accessibility, highly parallel arrays, low cell numbers, microfluidic functionality and three-dimensional cell culture may have important implications for diagnostics, drug development and basic research.

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