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Low cost microfluidic cell culture array using normally closed valves for cytotoxicity assay

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ABSTRACT

A reusable low cost microfluidic cell culture array device (MCCAD) integrated with a six output concentration gradient generator (cGG) and 4×6 arrays of microchamber elements, addressed by a series of row and columnar pneumatically actuated normally closed (NC) microvalves was fabricated for cell-based screening of chemotherapeutic compounds. The poly(dimethylsiloxane) (PDMS) device consists of three layers: fluidic, control and membrane which are held by surface contact and made leak-proof by clamping pressure. The NC valves are actuated by a thick PDMS membrane that was created by a novel method based on the self-assembly of PDMS pre-polymer molecules over a denser calcium chloride solution. The membrane actuated the valves reliably and particulates such as alumina particles (3 μm) and MCF-7 cells (20–24 μm) (2×10^5 cells/mL) were flowed through the valves without causing blockage or leakage and consequently avoiding contamination of the different cell culture elements.

The MCCAD was cast and assembled in a standard laboratory without specialist equipment and demonstrated for performing quantitative cell-based cytotoxicity assays of pyocyanine on human breast cancer (MCF-7) cells and assessed for toxic effect on human hepatocyte carcinoma (HepG2) cells as an indicator for liver injury. Then, the MCCAD was demonstrated for sequential drug combinatorial screening involving gradient generation of paclitaxel doses followed by treatment with aspirin doses on the viability of MCF-7 cells. The interaction between paclitaxel and aspirin was evaluated by using the Bliss independence predictive model and results showed reasonable agreement with the model. A robust, portable, easily fabricated and low cost device is therefore shown to conveniently carry out culturing of multiple cell lines for high throughput screening of anti-cancer compounds using minimal reagents.

1. Introduction

Drug discovery and development is a complex, lengthy and costly process which requires drugs to be tested for efficacy and toxicity in humans [1]. There is a high level of attrition of drug candidates, with only one in ten of those entering clinical trials becoming finally approved. Currently, drug development processes are mainly conducted using standardised macroscopic systems which incorporate automated analysis and robotics in a high-throughput manner, but these have drawbacks including high cost. Cell culture models are increasingly used to predict clinical response to drugs, this approach has the advantage of providing a more representative response to drugs than simple biochemical assays as well as obviating the need for whole animal testing which is lengthy, expensive and raises ethical issues. Cellular assays are often conducted in multi-well plates but suffer from a number of drawbacks including uncontrolled evaporation and inability to model multi-organ interactions.

The potential importance of microfluidic platforms in drug development has been widely recognised [2]. Microfluidic cell culture platforms integrated with key functional elements such as microvalves, mixers, gradient generators, detectors and heating elements are applicable for a variety of applications including a microchemostat [3]. They offer advantages in high-throughput screening owing to their ability to reduce amount of sample and reagent consumptions, precise control of fluids, automation, reduction of time and cost of conducting cell culture experiments and the potential to more accurately mimic the *in vivo* cellular microenvironment. Microfluidic systems have generally made use

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of large scale integration of pneumatically controlled microvalves pioneered by Quake and co-workers [4], these are broadly categorised as either the normally Open (NO) or normally Closed (NC).

Many of the previously reported cell culture array devices [5,6] use pneumatically controlled valves that are of the NO type [4]. These valves are costly to fabricate and require special flow channels with rounded cross sectional geometry. The lithographic mould manufacture for the NO valves requires a two stage process. In the first stage, SU-8 is exposed under a negative mask, the second stage uses a standard positive photoresist that requires accurate alignment under a positive mask and then a post-processing reflow process to achieve the rounded shape [4]. In the case of the NC valves there is only requirement for a single stage lithographic process using SU-8. For cell culturing, the flow requirements include loading of cells into the cell culture elements and periodic perfusion to feed the cells. In the case of multiple cell types, there is also a requirement for isolation of individual cell culture elements to prevent contamination. A NO valve type requires constant actuation to hold the valves shut and has limited fluidic channel aspect ratios since the membrane is required to stretch into the channel for sealing. The NC is fail safe and may be disconnected from the pneumatic system without leakage and contamination between the different cell culture elements. In the NC valves the thickness of the PDMS membrane has been shown to have little effect on the actuation pressure of the valve [7]. Thicker membranes are preferable since they are easier to handle, actuate reliably and easy to fabricate.

Here we report on the development of a PDMS MCCAD platform which integrates a two input cGG with six outlets that delivers into an array of 4×6 culture chamber elements addressed by row and columnar NC valves to direct and control fluid flow on the device. A novel low cost technique for producing membranes without the need for cleanroom facilities, for actuating the valves is described. In general, PDMS membranes are prepared by spin coating to a thickness down to 10 μm. The proposed method is based on the self-assembly of prepolymer molecules controlled by the surface tension difference between a salt solution and the PDMS prepolymer. We found this to be a simple and low cost method for the preparation of membranes down to 20 μm. The utility of the MCCAD was demonstrated for cell-based cytotoxicity and toxicity assays on MCF-7 cells and HepG2 cells.

2. Materials and methods

2.1. Design and fabrication of MCCA chip

The fluidic pathways of the MCCAD were designed in AutoCAD 2007 (Autodesk, USA), as shown in Fig. 1A. A 100 μm thick membrane was sandwiched between the two PDMS layers and separates the fluidic and control layers. There are interruption gaps of 100 μm on the floor of the fluidic microchannels (300 μm width, 200 μm depth) to define the valve seat. The control layer microchannels (200 μm wide, 200 μm depth) have an expanded terminal end to define the valve displacement chamber. The valve arrays (columnar and row) are controlled by two independent vacuum lines referred to as Group 1 and Group 2 (Fig. 1B) which are automated via LabVIEW 8.6 (National Instruments). Every microchamber is controlled by two pairs of microvalves to prevent cross contamination across the chambers and to allow loading of different cells on the cell culture chip (Fig. 1C). The fluidic and control layers were cast in PDMS (Dow Corning Sylgard 184) from an SU-8 master mould that had been made using standard lithographic procedures [8].

2.2. Preparation of PDMS membrane

The PDMS membrane was prepared using a simpler and cheaper novel method than the conventional way of spin coating, where a 10:1 (wt/wt) of the base and the curing agent were thoroughly mixed and degassed (30 min). The PDMS prepolymer was poured over a denser $CaCl_2$ solution contained in a Petri dish. The $CaCl_2$ solution (~15 mL, 400 g/L), filtered at room temperature, was transferred to a Petri dish (90 mm diameter) on a flat level surface. The less dense PDMS spreads evenly by natural reflow on the surface of the $CaCl_2$ solution creating a thin film. The volume of PDMS defines the thickness (t_m) of the film according to Eq. (1).

$$t_m = \frac{4v}{\pi d^2} \quad (1)$$

where v is the volume of PDMS, d is the diameter of the Petri-dish.

The developed PDMS membrane was cured at 60 °C for 2 h and removed gently from the salt solution followed by cleaning with 70% ethanol to remove any salts from the surface of the membrane.

2.3. Assembling of MCCAD

The MCCAD was packaged and assembled as shown in Fig. 1A using standard techniques [4]. The PDMS membrane (thickness 100 μm) was sealed to the control layer by a small clamping pressure. Small fluidic vias (1 mm diameter) were punctured on the PDMS membrane for the inlet and outlet ports. The non-structured side of the fluidic layer was sealed on a glass slide to provide support and the control layer pressure sealed to the PDMS membrane. The two composite layers were then aligned and pressure sealed under a magnifying glass. The structure was then clamped between PMMA sheets (thickness 3 mm) to seal it and support. Food colour dyes (red and green) were introduced in the assembled device to test the seal integrity of the valves and assess the performance of the gradient generator as described by Jeon et al. [9]. The presence of the air bubbles was avoided by ensuring that all the chip layers and potential openings were properly sealed to avoid leakage. The device was sealed by clamping the chip between two PMMA sheets and also the culture media was degassed using an ultrasonic bath.

2.4. Cell culture and cell seeding

MCF-7 and HepG2 cells were a donation from the Northern Institute for Cancer Research-Newcastle University. MCF-7 and HepG2 cells were separately cultured in Eagle's minimal essential medium (EMEM) supplemented with 2 mM L-glutamine, 1% non-essential amino acids, 10% FBS and 1% penicillin/streptomycin/amphotericin in 75 mm^2 flasks at 37 °C, 5% CO_2 humidified incubator until they reached 90% confluency. All cell culture media were purchased from Sigma Aldrich (UK). Cells were passaged a maximum of 4 times before being cultured on the cell culture array. Prior to loading cells, the MCCAD was sterilised by UV-radiation (30 min) followed by flushing the fluidic connections with 70% ethanol and rinsed with sterile phosphate buffer solution (PBS). Sterile fibronectin solution (100 μg/mL in PBS) was then used to coat the microchambers for 12 h at 4 °C. Cells were trypsinised, centrifuged, and the desired number of cells from each suspension were counted and used for seeding the MCCAD. MCF-7 and HepG2 cell suspensions (2×10^5 cells/mL) were seeded into the cell culture chambers when valves under Group 1 control line are open. After cell seeding, the Group 1 valve array was switched off, PTFE tubes removed and the inlet and outlet ports were sealed with silicone tape. The MCCAD was placed in an incubator and maintained at 37 °C and 5% CO_2 for 4–6 h for cell attachment. The attached cells were allowed to proliferate and