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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

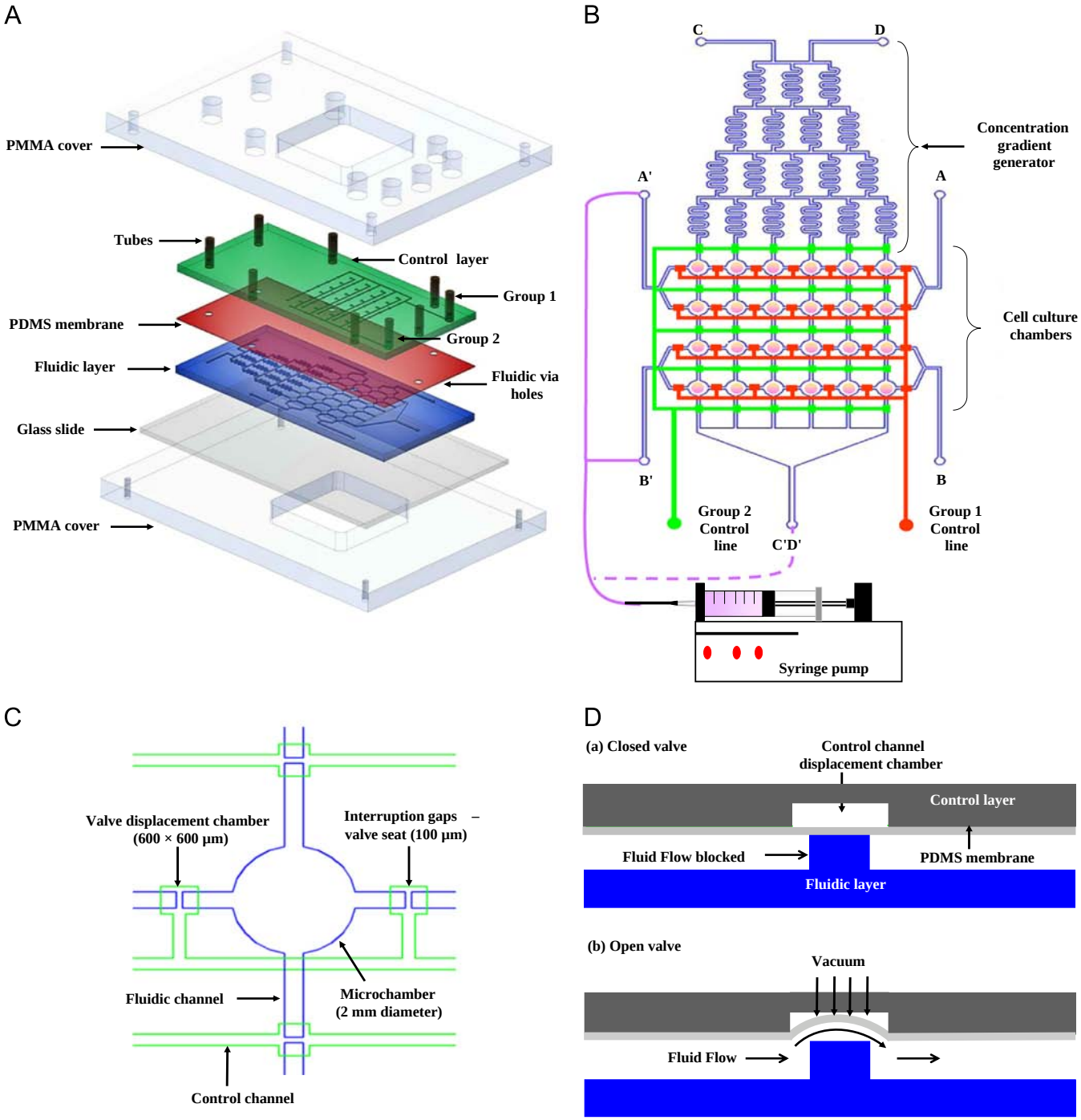

Fig. 1. Schematic of device assembling and system set up. (A) Exploded 3D representation of MCCAD. (B) Experimental set up. (C) Single cell culture chamber controlled by a set of four microvalves to prevent cell to cell communication and contamination. (D) Mechanism of opening and closing of a valve [10].

maintained for 24 h by flowing sterile degassed EMEM medium (3.5–5 μL/min) over a 2 min period through the cell culture device at 4 h intervals over a period of 24 h to feed the cells. This approach allowed metabolised media to be replenished periodically without exerting significant hydrodynamic shear stress on the cells.

2.5. Cytotoxicity and toxicity assays

After 24 h, MCF-7 and HepG2 cells were simultaneously exposed for 6 h to different concentrations of pyocyanine (Sigma Aldrich-UK) from the cGG. A stock solution of pyocyanine (100 μM) and EMEM were introduced into the inlets C and D of the cGG (Fig. 1B) and downstream culture chambers achieved pyocyanine concentration of (0, 20, 40, 60, 80 and 100 μM). The MCF-7 and HepG2 cells exposed to pyocyanine were treated with 2 μM calcien AM diluted in non-phenol red Dulbecco's Modified Eagle's Medium (DMEM) media for 30 min to assess cell viability. Calcien AM (Sigma Aldrich-UK) is a non-fluorescent dye that permeates easily in live cells and is hydrolysed intracellularly by esterases enzymes in the cytoplasm to calcien which produces green fluorescence. Cell imaging was carried out with a TE-2000 Nikon fluorescence microscope equipped with a low light intensity monochromatic CCD camera using the 4× and 10× objectives.

Viable cells were enumerated by counting the green fluorescent cells. Cell viability after pyocyanine treatment was expressed as percentage of live cells, in each chamber treated with the chemical relative to the number of live cells in a control.

Drug combination assays were conducted by exposing MCF-7 cells seeded in the microchamber elements of the MCCAD to a sequential combination of paclitaxel and aspirin (Sigma Aldrich-UK) doses. Aspirin and paclitaxel were dissolved in dimethyl sulfoxide (DMSO) and further diluted with Eagle's minimal essential medium (EMEM) to final concentrations of 10 mM and 1.2 μM respectively. Initially MCF-7 cells were exposed to concentrations of paclitaxel (0, 0.2, 0.5, 0.7, 0.9 and 1.2 μM) from the cGG for 6 h followed by sterile phosphate buffer rinse. The MCF-7 cells were then exposed for 6 h to concentrations of the second drug aspirin (0, 2, 4, 6, 8 and 10 mM), followed by assessing cell viability with calcien AM as described for the pyocyanine assays.

Conventional 96 culture well plates were seeded with the same cell density for MCF-7 and HepG2 cells as for the MCCAD and incubated at 37 °C and 5% CO_2 for 24 h followed by challenging cells with the respective drug doses. Cell viability was assessed as previously described for the MCCAD.

3. Results and discussion

3.1. Design of the MCCAD

The integration of various commonly used fluidic components (gradient generator, and microvalves) as part of the MCCAD offers several advantages including reducing the number of mixing and pipetting steps and consequently experimental error. Normally PDMS devices are permanently bonded using either oxygen plasma or curing, the MCCAD platform uses surface contact and a clamping force to provide a seal for leak-proof operation. The reversible sealing between the layers allows the device to be reused and with the different parts being able to be cleaned or replaced. A further advantage is that the alignments of the three layers is not time constrained and so are less susceptible to damage, unlike when oxygen plasma bonding is used. A drawback of PDMS based devices is that they cannot be manufactured at high volume so the approach described here reduces the number of devices that need to be fabricated reducing effort and cost of device fabrication. In large scale integrated microfluidic systems, microvalves are required to be leak-proof (tight seal integrity), scalable and carry out multiplexing for individually addressing each element of cell culture array [11,12]. The NC valve design is fail safe in that when no vacuum is applied they are closed which prevents contamination between the adjacent culture chambers. Moreover, the NC valve design is easy to integrate with complex microfluidic platforms and does not require pressure to close them, thus making it highly portable without leaking or mixing of cell culture chamber contents. The opening pressure of the NC valve depends on the thickness of the membrane, the mechanical and bulk properties of the membrane material, adhesion forces between the membrane and the valve stop and dimensions of the vacuum well.

The cells used in this study were between 20 and 24 μm and to avoid clogging within the device we choose the fluidic architecture to have larger channel dimensions of 300 μm × 200 μm. As a consequence a larger displacement chamber of 600 μm × 600 μm was used which allows a thicker PDMS membrane. Different membrane thicknesses (50–125 μm) were investigated for their effect on the opening of the valve and a linear relationship was observed between the membrane thickness and the opening pressure. A membrane thickness of 100 μm was chosen for ease of handling and a vacuum of 80 kPa was required to fully open the valve. The NC type valves have been less well studied within the literature for tolerance to particulate flow. The valves were first tested for robustness to particulates using different sizes and concentrations of particles. A suspension of 0.01 g/mL alumina particles with particle diameter of 3 μm were found to flow through the valves. Subsequently, testing of clogging arising from human cells was tested using trypsinised suspensions of MCF-7 cells (20–24 μm) (2×10^5 cells/mL) and these were also found to flow through the valves and not clog the microfluidic channels or disrupt the valve operation.

A standard gradient generator was designed to output 0–100% concentration solutions in 20% steps [9]. The performance of the

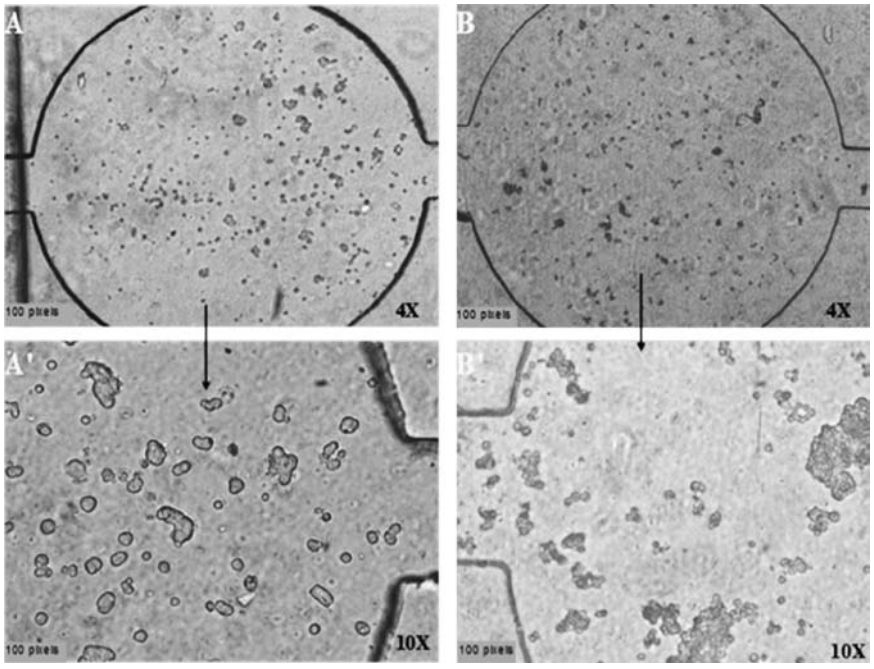

Fig. 2. Seeded cells attached and localised inside the MCCAD microchambers. AA′ MCF-7 and BB′ HepG2.

cGG was tested using a standard stock solution of pyocyanine (100 μM) and EMEM with the final concentrations of pyocyanine being determined by absorbance using linear interpolation with a standard calibration curve and results compare well with literature reports [13].

3.2. Cell culture in the MCCAD

We demonstrated the ability of the MCCAD to sustain cell proliferation by culturing, MCF-7 and HepG2 within the different culture chamber elements without compromising the integrity of each cell type through contamination. Fig. 2 shows representative microscopic images of seeded MCF-7 and HepG2 cells in the respective culture chamber elements. The results showed that both the MCF-7 and HepG2 cells proliferated well in the cell culture environment with good morphology. These findings demonstrated the suitability of the substrate material, method of periodic perfusion of culture media and that the general experimental approach was appropriate for cell growth. A fed-batch method was used to periodically replenish culture media and removal of cellular waste products every 4 h. This approach has the advantage of shielding cells from being exposed to the effects of high hydrodynamic shear stress gradients which may be produced during continuous perfusion of media and adversely affect cellular behaviour rendering them unsuitable for experimental work. In contrast to bacterial cells, mammalian cells do not have a cell wall and are therefore susceptible to the effects of hydrodynamic shear stress. The use of PDMS as a material substrate for the MCCAD was found to be suitable for performing long term cell culturing experiments. PDMS has a number of desirable properties including non-toxicity, optical transparency and permeability to O_2 and CO_2. The relatively high permeability allows exchange of gases between the external atmosphere of the chip and medium in the culture chambers and consequently supports proliferation of the cells. PDMS is, however, susceptible to non-specific protein adsorption which can result in depletion of protein levels within the medium and affect the consistency of culture conditions and cellular behaviour [14]. A variety of pharmaceutical compounds can be adsorbed on the PDMS material and this can vary depending on factors such as pH and the ions in solution [15]. Sorption of the cytotoxic agent by PDMS will result in lowering of the cytotoxicity concentration which further complicates the analysis. Interference from sorption effects are reduced by coating the cell culture wells with fibronectin molecules to render them hydrophilic and biocompatible for cell adhesion.

3.3. Cytotoxicity and toxicity assays

MCF-7 and HepG2 cells were used as simple models for breast cancer tissue and the liver organ respectively to mimic the physiological *in vivo* conditions of the human body on cytotoxicity tests and chemical toxicity. Previous studies have shown that pyocyanine induces toxicity in both prokaryotes and eukaryotes cells by engaging in non-enzymatic redox reactions which generates reactive oxygen species (ROS) such as O_2^- and H_2O_2 [16,17]. These ROS species deplete cells of NADH, glutathione and other oxidants causing oxidative stress leading to cell death due to DNA damage [18].

The efficacy of pyocyanine against MCF-7 cells was estimated from the LC_{50}, concentration of the cytotoxic agent at 50% cell viability. The LC_{50} for pyocyanine on MCF-7 cells was 60 ± 3 μM for the MCCAD and 51 ± 3 μM for 96 culture well plates. Some difference of this value in comparison with the microfluidic cell culture array can be expected since the microfluidic cell culture device has periodic perfusion of nutrients and the constant removal of waste. Conventional culture well plates operate under static conditions with uncontrolled liquid evaporation due to relatively high surface area to volume ratio which can result in poorly defined culture conditions.

Toxicity screening assays for drugs provide crucial information on preclinical studies in assessing the safety aspects of the potential drug candidates [19]. The MCCAD was used to model the response of HepG2 liver cells to the toxic effect of pyocyanine. HepG2 cells were used as the model cell line because they are highly differentiated and display similar genotypic features typical of primary hepatocytes [20]. Experimental results showed that approximately 70% of HepG2 cells exposed to pyocyanine concentration of 100 μM, in both the microfluidic device and the culture well plates, were still adherent and viable. The resistance of HepG2 cells to pyocyanine could be due to the higher levels of peroxidase and catalase enzymes found in liver hepatocytes [21]. The actual causes of resistance to pyocyanine and its mode of action is an area that requires further investigation.

3.4. Drug combination treatment

The microfluidic cell culture array platform was used to investigate the effect of exposure of chemotherapeutic drugs paclitaxel and aspirin, individually and as a sequential combination, on the viability of MCF-7 cells. MCF-7 cells were cultured in the MCCAD and exposed to the treatment with doses of paclitaxel and aspirin as single drugs acting alone and sequential combination. Cell numbers were manually counted and viability was expressed as percentage of live cells, in each chamber treated with the drug combinations relative to the number of live cells in the control. The mean values for cell responses to the combination of paclitaxel and aspirin doses and control conditions were fitted on linear regression curves. The fitted concentration–response curves were used to determine the concentrations of the drug combinations. Statistical significance was evaluated by one way analysis of variance (ANOVA) using student's t test. $P < 0.05$ was considered as statistically significant.

Fig. 3 shows the fluorescent images of MCF-7 cells after exposure to either paclitaxel or aspirin alone and their sequential combination activity.

The results in Figs. 3B and C and 4A showed that the exposure of MCF-7 cells to concentrations of paclitaxel alone was dose dependant. The LC_{50} value for paclitaxel alone on MCF-7 cells was 0.63 ± 0.04 μM and 0.55 ± 0.05 μM for 96 culture well microplates. Paclitaxel inhibits and disrupts the microtubule network organisation in neoplastic tissues resulting in the arrest of the G2/M cell cycle and the inability of cells to properly complete mitosis [22].

The exposure of MCF-7 cells to concentrations of aspirin alone induced loss of viability in a dose dependent manner (Figs. 3D, G and 4A). MCF-7 cells exposed to 2 mM of aspirin alone gave a modest 5% reduction in the cell viability (Figs. 3D and 4A). At higher doses (8–10 mM) of aspirin, above the therapeutic plasma concentration, there was significant reduction of around 40% on the cell viability (Fig. 4A). The LC_{50} for aspirin alone on MCF-7 cells in the MCCAD was found to be 9.8 ± 1.0 mM (Fig. 4A) and 6.9 ± 0.3 mM for 96 culture well microplates. A LC_{50} value of 6.6 mM has been previously reported for aspirin on human MCF-7 cells when exposed to the drug for 24 h in 96 culture well microplates [23]. The action of aspirin against adenocarcinomas is attributed to its ability to inhibit cyclooxygenase enzymes COX-1 and COX-2 mostly expressed in tumour cells [24]. Aspirin facilitates the reduction in angiogenic factor secretion and production of prostaglandins in tumour cell activity, thus restricting tumour growth [25].

The use of combination of drugs, for treatment of cancer and infections, offers the potential to identify a lower dose for

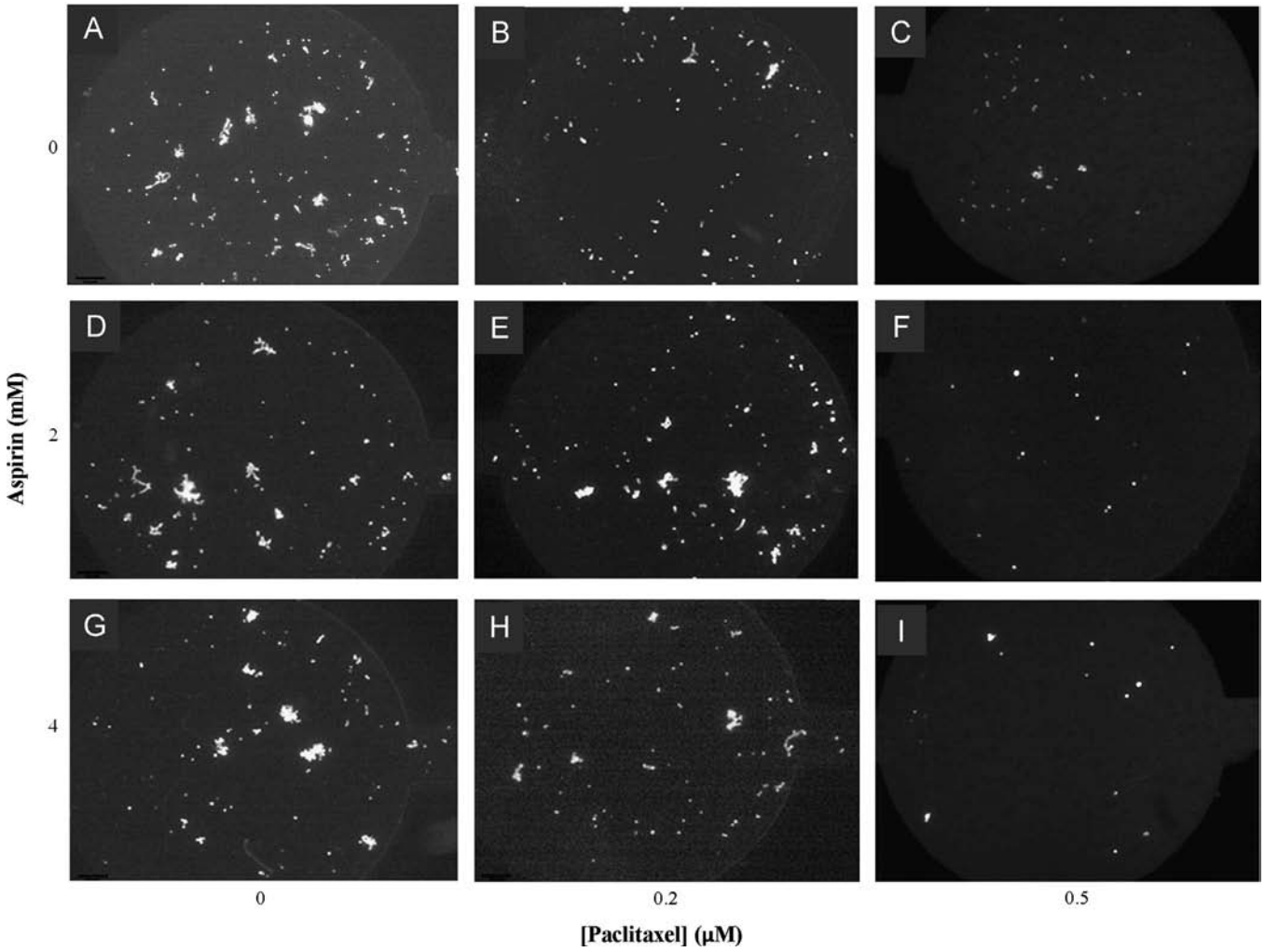

Fig. 3. Selected fluorescent images of MCF-7 cells taken at (4 ×) after sequential treatment with paclitaxel and aspirin. MCF-7 cells were first exposed to six concentrations (0, 0.2, 0.5, 0.7, 0.9 and 1.2 μM of paclitaxel for 6 h followed by exposure to six concentrations (0, 2, 4, 6, 8 and 10 mM) of aspirin for further 6 h and assessed for viability with calcien AM fluorescent dye.

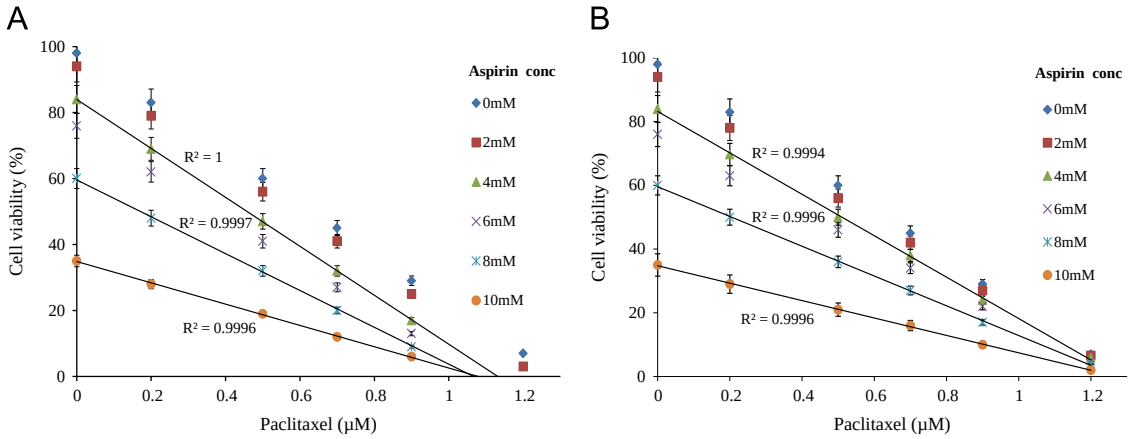

Fig. 4. Response of MCF-7 cells after sequential treatment with paclitaxel and aspirin in the MCCAD. (A) The regressed data plot showing the experimental sequential combination effect of paclitaxel and aspirin at varying concentrations on cell viability. (B) Bliss Independent Predicted model showing the effect of paclitaxel and aspirin at varying concentrations on MCF-7 cell viability. The legends on A and B represent the concentration of aspirin (0, 2, 4, 6, 8, 10 mM) in combination with the concentrations of paclitaxel. Data shown represents the mean of three independent experiments. Error bars are plus or minus standard deviation.

maximising cancer cell death and eradication of infections with less side-effect. Combination therapy reduces the risk of drug resistance and discovery of synergies in a variety of diseases including tuberculosis, malaria, HIV AIDS and metastatic breast cancers [26]. Ovarian and breast cancer cells show some resistance to paclitaxel treatment alone and so combination therapy is attractive [27]. The experimental effect of paclitaxel and aspirin combination on the viability of MCF-7 cells is seen qualitatively from the fluorescent images in Figs. 3 and quantified in 4A. In Fig. 3A, MCF-7 cells exposed to the control/no drug treatment

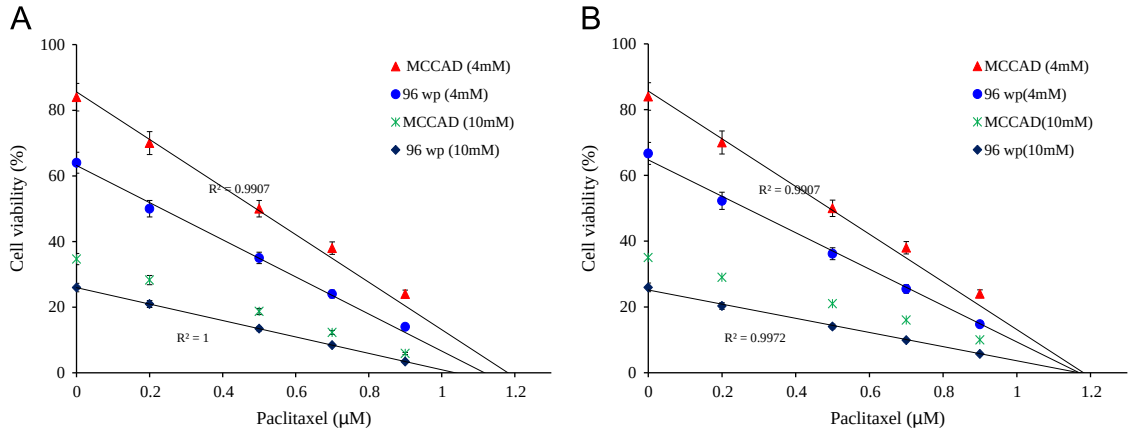

Fig. 5. Comparison of MCF-7 cells after sequential treatment with paclitaxel and aspirin in the MCCAD and 96 culture well plates (96 wp) at selected concentrations. Sequential treatment of MCF-7 cells with (0.0, 0.2, 0.5, 0.7, 0.9 and 1.2 μM) of paclitaxel for 6 h followed by exposure to 4 mM and 10 mM of aspirin for 6 h. (B) Bliss Independent Predicted model showing the effect of paclitaxel doses (0.0, 0.2, 0.5, 0.7, 0.9 and 1.2 μM) followed by exposure to 4 mM and 10 mM of aspirin on MCF-7 cell viability. Data shown represents the mean of three independent experiments. Error bars are plus or minus standard deviation.

conditions (0 μM paclitaxel and 0 mM aspirin), proliferated well and had higher viability. The exposure of MCF-7 cells to 0.2 μM of paclitaxel followed by 2 mM of aspirin showed 21% loss of cell viability than 17% and 6% when each drug was administered alone respectively (Fig. 3E).

Interestingly, it can be seen that one combination of 0.46 ± 0.07 μM of paclitaxel with 4 mM of aspirin gives an LC_{50} which is at lower drug concentrations than is the case for the use of individual drugs (Fig. 4A). The drawback of high drug concentrations of the individual drug compounds is that they may cause damage to secondary organs such as the liver or kidneys. The interaction between paclitaxel and aspirin was evaluated by using the Bliss Independence model [28,29]. Within the experimental space of this study, the data showed reasonable agreement with the model and the effect of paclitaxel and aspirin on cell viability appears to be cumulative. The predicted cumulative effect of paclitaxel and aspirin are shown in Fig. 4B and obtained from the experimental individual effects of each drug given in Fig. 4A. As an example, a paclitaxel concentration of 0.2 μM alone reduces cell viability to 83% whilst aspirin at 4 mM concentration alone reduces cell viability to 84% (Fig. 4B). The predicted cumulative effect of the paclitaxel and aspirin, at these concentrations, will be $0.83 \times 0.84 = 0.697$ or 70% (Fig. 4B). It can be seen that the experimental observations in Fig. 4A compare well with those predicted in Fig. 4B.

The performance of the microbioreactor array platform was compared with the conventional 96 culture well plate at selected concentrations. Fig. 5A shows the comparison of MCF-7 cells exposed to paclitaxel concentrations (0, 0.2, 0.5, 0.7, 0.9 and 1.2 μM) followed by exposure to 4 mM or 10 mM of aspirin in the MCCAD and the 96 culture well plate (96 wp). MCF-7 cells exposed to the drug combinations treatment in the MCCAD consistently showed more growth than in the 96 culture well plates and so provided a better platform for detailed cell-based assay investigation. The LC_{50} values for the combination of paclitaxel doses followed by exposure to 4 mM aspirin for the MCCAD and the 96 culture well plates were 0.46 ± 0.02 μM and 0.26 ± 0.01 μM respectively. In general, a similar trend was also obtained with other combinations (results not shown). It can be seen that the experimental observations in Fig. 5A for the MCCAD and the 96 culture well plate compare well with those predicted in Fig. 5B. The different response of MCF-7 cells in the MCCAD and 96 culture well plates to the combined drug treatment could be due to the absorption or adsorption of the drug molecules by the substrate material (PDMS) in the MCCAD, which could lower the concentration of the drugs giving rise to a reduced cytotoxicity response.

4. Conclusion

An integrated microfluidic 24 element cell culture array using NC valves was successfully demonstrated for low cost cell-based screening of chemotherapeutic compounds including screening of drug combinations. The device was cast on an SU-8 mould in a standard laboratory without specialist equipment. A simple NC valve design with a thick membrane and reversible sealing which allowed the device to be disassembled and either cleaned for re-use or the parts replaced. The NC valves were shown to be robust and reliable in operation and allowed the device to be disconnected and moved between for different stations for different operations. The system has been shown to be viable as a tool for *in vitro* clinical drug development. Further work will involve more detailed investigation of the effective dosage range as well as validating further possible combinations of chemotherapeutic compounds and their interaction with cell culture models that better recreate the complex *in vivo* cellular microenvironment, such as 3D cell culture or spheroid formats.

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