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Pumpless platform for high-throughput dynamic multicellular culture and chemosensitivity evaluation

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Abstract

We report here a novel pumpless, 96-well plate-based platform for high-throughput dynamic multicellular culture and chemosensitivity evaluation. A gravity-driven flow strategy was developed to generate and sustain the flow rate of culture medium within 10% in the platform's 20 culture chambers. The ability of the platform to generate and sustain the medium flow was demonstrated by computational simulation, flow visualization, and ascertaining the previously known effect of flow-induced shear stress on the stimulated osteogenic differentiation of osteoblasts. The high-throughput utility of the platform was demonstrated by in situ cell staining and high content screening of chemosensitivity assays of multiple myeloma and osteoblast co-cultures. Endpoint characterization and data analyses for all 20 culture chambers required less than 1 hour.

Graphical Abstract

We report here a 96 well-plate based pumpless platform for high-throughput dynamic multicellular culture and chemosensitivity evaluation.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here].

Conflicts of interest

There are no conflicts to declare.

Introduction

A major challenge with evaluating the drug response of patient-derived tumor cells is the significant lack of clinically relevant, high-throughput and inexpensive *in vivo* and *in vitro* models. For example, patient-derived xenograft models of multiple myeloma (MM) and metastatic breast and prostate cancers are complicated, inconsistent, time-consuming, and costly to use^{1–3} because human tumor cells rarely metastasize to animal bones due to species-related differences. Also, it has been difficult to keep the viability of patient-derived MM cells (PMMC) over several hours using conventional cell culture techniques.⁴

Recently, major efforts have been made to develop *in vitro* MM models for drug evaluation.^{5–14} Of note, 3D engineered hydrogel with stromal cells was commonly used as biomimetic scaffolds for PMMC, e.g., fibrin gel with endothelial cells and bone marrow stromal cells,¹⁰ hydrogel modified by mesenchymal stem cells,⁵ and collagen type I with bone marrow stromal cells.⁸ Also, microfluidic culture devices were used to apply shear flow and enable the viability of PMMC.^{11,12} These studies suggested that replicating physiologic relevant bone marrow micro-environments is critical for *in vitro* PMMC maintenance.

We also developed and demonstrated a 96-well plate-based perfusion device that can be used to maintain the viability and proliferative capacity of PMMC over 3 weeks.¹⁵ We found that: (1) the adhesion of PMMC to osteoblasts (OSB) and the long-term viability of OSB are critical factors for the *ex vivo* survival of PMMC and (2) perfusion flow rate can be optimized to increase the long-term viability of PMMC.^{15–17} These findings support the culture platform's capability to recapitulate critical environmental factors associated with the survival of patient tumor cells. However, the high-throughput operation of this dynamic culture platform has been limited since the use of external pumps and tubing connections is labor-intensive and error-prone to cause bubble formation and fluid leakage.^{18–20}

Herein, we completely redesigned our culture platform to facilitate its high-throughput use by implementing a gravity-driven, pumpless flow control strategy (Fig. 1). Pumpless microfluidic culture has been increasingly pursued to reduce the complexity and difficulty of fluidic management. Hydrostatic pressure,^{21,22} capillary force,²³ and surface tension²⁴ are commonly utilized as passive driving forces to generate fluid flow. We selected to use gravity-based medium feeding because: (1) it can be implemented in a high-throughput manner in synchronization with the automated operation of a commercial plate dispenser and (2) it has been previously demonstrated by Yuen *et al.*¹⁸ in a 96-well plate format for dynamic cell culture through the innovative use of a flow rate-controlling membrane between wells.

Device Design and Features

As shown in Figs. 1a and 1b, the pumpless platform consists of a 96-well plate based microfluidic device and a programmable rocking device which are housed in an incubator. The microfluidic device contains 20 culture chambers with each chamber occupying 4 wells (Fig. 1c). The inlet and outlet wells function as the reservoirs for culture medium and to generate hydrostatic pressure differential between the wells (P). Cells are plated through

the cell seeding port. The device is designed to enable imaging of cell cultures via high content screening (HCS) or conventional microscopy. Fig. 1d, obtained with HCS, shows OSB and MM cells co-cultured in the culture chamber and stained *in situ* with viability stains prior to imaging. HCS compatibility is an important feature of the culture platform, as it has become a core technique in the early stage of drug discovery.²⁵ It enables rapid collection and analysis of images and extraction of useful information at a rate that far outpaces conventional microscopy and flow cytometry.

Fig. 2 shows the components of the microfluidic device with detailed illustrations of its design features. As illustrated in Fig. 2b, the seeding port was designed to allow the insertion of a 200 μL pipette tip into the port and therefore directly pipette cells to the culture chamber. Prior to seeding, the chamber is conditioned with culture medium. Cells are directed to the culture chamber due to the high fluid resistance present in the left channel. The culture chamber is connected to the cell seeding port by a microchannel of 0.5 mm width and 0.137 mm depth. The culture chamber is hexagonal in shape with the lateral surface area of 38.3 mm², which is comparable to that of a typical 96 well plate (38.5 mm²). The thickness of the culture chamber is 0.137 mm.

In order to control the medium flow rate (Q) through the culture chamber,²⁶ a 0.4 μm pore size polycarbonate (PCTE) membrane is imbedded, as a flow rate controller, in the inlet well (Fig. 2c). Since the flow is laminar, the Hagen-Poiseuille equation can be used to calculate Q that is generated by P :

$$\frac{\Delta P}{Q} = \frac{32\mu L_m}{\epsilon A_m d_m^2} \quad (1)$$

where P is the pressure drop between the medium reservoirs, μ is the dynamic viscosity of the medium (0.7 mPa.s under the incubator condition), ϵ is the porosity of the membrane (10% for the 0.4 μm PCTE membrane), A_m is the surface area of the membrane (1.77 mm²), L_m is the thickness of the membrane (10 μm), and d_m is the pore diameter of the membrane (0.4 μm). For example, Q of 0.5 $\mu\text{L}/\text{min}$ can be generated in the culture chamber if P is 67 Pa. The corresponding wall shear stress (τ) at the bottom surface of the culture chamber is calculated to be 0.4 mPa using:

$$\tau = \frac{6\mu Q}{h_c^2 w_c} \quad (2)$$

where h_c is the height of the culture chamber (0.137 mm) and w_c is the chamber width (5.35 mm).

Computational simulation results, summarized in Fig. 3, support the analytical solutions, i.e., the pressure drop of 67 Pa can be used to generate the flow rate of $\sim 0.5 \mu\text{L}/\text{min}$ and τ of ~ 0.4 mPa in the culture chamber. The simulation results were obtained using the computational fluidic dynamic module of Comsol 5.0, as detailed in Supplementary

Information 1. As can be seen from the pressure drop contours in Fig. 3a, the major pressure drop occurs when the medium goes through the PCTE membrane in the inlet well. Fig. 3b shows that shear stress distribution was relatively uniform with the shear stress of 0.4 mPa on most part of the culture chamber.

Fig. 4 illustrates how the tilting degree of the rocking device can be controlled with time to control Q to be within 10% during culture. Since the medium level difference decreases continuously with time (t) under this gravity-based approach, time-dependence on the medium level difference (h) and the medium flow rate (Q) in the culture chamber can be accounted for using:

$$\Delta h = \Delta h_0 e^{-2Q_0 t / \Delta h_0 A} \quad (3)$$

$$Q = Q_0 e^{-2Q_0 t / \Delta h_0 A} \quad (4)$$

where h_0 is the initial medium level difference, A is the cross-sectional area of a 96-well, Q_0 is the initial flow rate (0.5 $\mu\text{L}/\text{min}$). Note that the residence time of the medium in the culture chamber, based on Q_0 , is ~ 17 min.

In order to maintain Q within 10%, the tilting angle of the rocking device is increased from 14.4 to 15.5 degree after 20 minutes into culture (Fig. 4a). This 1.1 degree increase is repeated for two more 20-minute intervals. Then, the tilting position is reversed to -11.1 degree to re-establish h_0 and the incremental tilting angle changes are repeated. Fig. 4b shows that this tilting control strategy results in bi-directional fluid flow with Q in the range of 0.46 to 5 $\mu\text{L}/\text{min}$. The overall time spent for one tilting cycle is 120 min. During this time, ~ 60 μL of the medium passes through the chamber.

Fig. 2d illustrates the surface pattern used to: (1) hold the medium in the cell seeding port by surface tension during dynamic culture and (2) prevent evaporation of liquid in the medium reservoir ports during device pre-treatment and static culture. After seeding cells, several hours are needed for the cells to settle and adhere without fluid flow. During this time, the medium is only present in the culture chamber and the microfluidic passages, but not in the reservoirs. When the medium dries in these small passages and the medium is subsequently added to the reservoirs, bubbles are generated in the microfluidic passages preventing medium flow. In order to mitigate this problem, the surface of the seeding and reservoir ports is patterned. Basically, a digital-cut pressure sensitive adhesive (PSA) tape (Arcare 90106®, Adhesive Research) is adhered with a ring structure exhibited on each port surface. With the patterned PSA layer, a stable medium droplet is created and maintained on the port surface for several hours (Fig. 2d). The stable droplet also keeps the medium from evaporating. Furthermore, during culture, the patterned surface of the cell seeding port ensures that the medium does not exit through the seeding port.

Fig. 2e shows that the top of the culture chamber is covered with a transparent polyester (PETE) membrane with the thickness of 12 μm and the pore size of 0.4 μm . This membrane is used to hold the medium within the chamber during cell seeding and culture. At the end of culture, cell washing and staining are carried on *in situ* by directly pipetting and aspirating solutions through the membrane. Liquids above and below the membrane are then mixed and equilibrated by diffusion.

Device Fabrication

As shown in Fig. 5, the microfluidic device was assembled with a commercial polystyrene bottomless 96 well plate (Greiner Bio-One™), a polydimethylsiloxane (PDMS) layer, three PSA layers, and a glass plate (Fig. 5a). The PSA layers were digital-cut into different patterns using digital craft cutter (Silhouettet™ CAMEO). As previously demonstrated²⁷ the use of the digital cutter enabled the overall fabrication of the experimental devices to take less than 2 hours including PDMS preparation (degassing and curing) and patterning of the PSA layers.

As shown in Fig. 5a, the first PSA layer, directly adhered to glass plate, was used to connect the wells and define the lateral shape and dimensions of the culture chamber. The second PSA layer is used to adhere and seal: (1) the PCTE membrane (Sterlitech™ PCT0447100) that controls the flow rate of medium in the culture chamber and (2) the PETE (Sterlitech™ 1300017) membrane on the culture chamber which is used to hold the medium within the culture chamber during cell seeding and culture. The PCTE membrane was also cut using the digital cutter. The membrane was placed between two thin plastic layers. The sandwiched membrane and layers were taped to the cutting matt and cut. This procedure was useful to protect the membrane from adhesive present on the cutting matt and enable the fabrication of round membrane pieces without any tear or rupture (Fig. 5b). The same procedure was used to prepare the PETE membrane. The third PSA layer was used to pattern the surface of the medium reservoirs and the cell seeding port.

Because of the large and rigid glass plate used for the device bottom, we found it difficult to seal the well plate only with the PSA layers. Introducing the PDMS layer (Fig. 5a) ensured to seal the microfluidic passages to be leak-free. Also, the holes in the PDMS layer were used to guide liquid injections using 200 μL pipette tips during various stages of cell culture, washing, and staining. The holes in the PDMS layer were made using biopsy punches (Miltex™) of 1.5 mm and 6 mm diameter. The PDMS layer was plasma treated before adhesion to the adjacent PSA layers.

Flow Visualization

As shown in Fig. 6, 20 μm polystyrene microbeads (Polybead® Polystyrene Microspheres) were used to visualize flow through the fabricated device. In brief, we filled the culture chamber with phosphate-buffered saline (PBS), pipetted 50 μL of PBS into both medium reservoirs, and then added 10 μL of the microbeads into one of the wells. The surface tension and buoyancy force kept the microbeads floating on the medium surface. The device was left in the incubator for 30 minutes for the liquid to equilibrate and reach 37°C and then placed

under a microscope (Nikon Ti). The bright-field images of the floating microbeads were captured as shown in Fig. 6b. The flow was generated by adding 260 μL of pre-warmed PBS into the inlet well (Fig. 6a). The vertical position of the microbeads, i.e., the height of the liquid, was measured as a function of time using the microscope (Fig. 6b). Fig. 6c shows that the position of the microbeads could be resolved within 20 μm by adjusting focus. The experimentally measured h values matched well to those calculated using the design equations (Fig 6d). In 20 minutes, h increased 250 μm in the outlet and flow rate decreased from 0.5 $\mu\text{L}/\text{min}$ to 0.46 $\mu\text{L}/\text{min}$. As mentioned in the device design section, a rocking platform was used here to reset the liquid height difference between inlet and outlet, to maintain the flow rate within 10%.

Cell Culture

MM.1S (MM cell line, ATCC®CRL-2974) and hFOB 1.19 (human OSB cell line, ATCC®CRL-11372) were purchased from ATCC. MM.1S cells were cultured in high glucose RPMI-1640 medium supplemented with 15% fetal bovine serum (FBS), 2.5 mM of L-glutamine and 1% penicillin/streptomycin. hFOB 1.19 cell medium consisted of 1:1 mixture of Ham's F12 Medium Dulbecco's Modified Eagle's Medium, with 2.5 mM L-glutamine, 10% FBS and 0.3 mg/ml G418 (Sigma-Aldrich). hFOB 1.19 and MM.1S cells were maintained and propagated in a 5% CO_2 incubator at 34°C and 37°C respectively. Co-culture experiments were conducted at 37°C. A 1:1 mixture of MM.1S and hFOB 1.19 growth media was used for co-culture. All cell lines were periodically checked for mycoplasma using MycoAlert™ Mycoplasma Detection Kit (Lonza). Authentication of cell lines was performed by STR DNA profiling analysis conducted by the Molecular Resources Facility at Rutgers University. Cell populations were frozen after 3 passages from the time of initial receipt and growth and were discarded after 20 passages.

Dynamic Culture of OSB Cells

We used the human OSB cell line hFOB 1.19 to validate that the pumpless culture device: (1) supports long-term OSB culture and (2) can generate flow-induced shear stress that promotes osteoblastic development. Studies²⁸ have shown that flow-induced shear stress could stimulate OSB proliferation and activity. We have also established the importance of perfusion in maintaining the long term viability of OSB and PMMC.¹⁶

Before introducing OSB cells, the culture chamber was sterilized with 70% isopropyl alcohol for 30 minutes, and functionalized with fibronectin (Corning™ Fibronectin, Human). Briefly, 20 μL fibronectin solution (150 $\mu\text{g}/\text{mL}$, which equals to 2 $\mu\text{g}/\text{cm}^2$) was pipetted into the chamber from cell seeding port to fill up the chamber. Then 5 μL solution was also added onto each port to prevent evaporation during coating. The device was left in 37°C for 1h, then washed with PBS and OSB growth medium. 10 μL OSB suspension with a cell density of 2 million/mL were introduced through the cell seeding port (1 $\times$ 10⁴ cells/chamber), static culture for 4 h to let them adhere. We observed that there were just a few cells occasionally settled in and attached to the channel between the seeding port and culture chamber. We attributed this behavior to the higher shear stress in the channel than in the culture chamber (4 mPa vs. 0.4 mPa). Then, 100 μL medium was added to both reservoirs,

and the device was placed on a rocking platform in a 34°C incubator to start the dynamic culture. Medium was changed every two days.

The rocking platform was assembled using a commercial rotator (R4040, Benchmark Scientific) and by connecting its stepper motor to a microcontroller (Arduino Uno) and a driver (A4988) on a computer numerical control (CNC) shield. The tilting angle was set to 14.4 degree initially to generate a 6.7 mm medium level difference between the inlet and outlet wells. According to the tilting control strategy in Fig. 4, a bi-directional medium flow was generated during culture with the flow rate maintained within 10%. Another pumpless device was used as static control. After introducing the cells, 200 μ L of medium was pipetted carefully onto the PETE membrane present on top of the culture chamber. The PETE membrane allowed the medium to diffuse into the chamber, and no flow was generated because the medium was held in the ports from entering into the well.

Cell viability and alkaline phosphatase (ALP, a measurement of osteoblastic activity) were determined after 4-day dynamic culture of OSB. Fluorescent images are shown in Fig. 7a, calcein AM (green) labels live hFOB and displays cell morphology. Most hFOB cells were oriented along with the flow direction under dynamic culture, whereas under static culture, cells show random orientation.

Cell nuclei were stained with 5 μ g/ml Hoechst (Hoechst 33324). LIVE/DEAD Viability/Cytotoxicity Kit (0.1 μ M calcein AM & 0.1 μ M EthD-1; Thermo Fisher Scientific) was used to visualize live and dead cells. Fluorescence images were captured using an inverted microscope (Nikon Ti-E) and analyzed using ImageJ 1.49v. OSB were significantly more viable after 4 days of dynamic culture ($93.29\% \pm 0.68\%$) than under static culture conditions ($86.76\% \pm 2.93\%$) [$*P < 0.05$, Fig 7a].

For ALP activity, we used TRACP&ALP assay kit (TaKaRa #MK301). It detects ALP through the use of pNPP (p-nitro-phenyl phosphatase) and it is quantified by measuring the absorbance of the reactant at 405 nm. Fig. 7c shows that the ALP activity was significantly higher under dynamic culture (0.39 ± 0.07) than under static culture (0.27 ± 0.02) [$*P < 0.05$, Fig 7b]. These results are similar to those established using our previously designed pump-fed culture platform.¹⁷ Therefore, the results validate that the pumpless device can be used to maintain viable OSB, generate flow-induced shear stress, and promote the osteoblastic differentiation of these cells.

Chemosensitivity Evaluation of MM Cells through *In Situ* Cell Staining and HCS

The high-throughput capability of the pumpless culture platform was evaluated by studying the response of MM.1S cells to bortezomib at four different concentrations (0 nM, 5 nM, 10 nM, 20 nM). Bortezomib is a proteasome inhibitor that induces MM cell apoptosis.²⁹ Before introducing MM.1S cells, OSB were dynamically cultured for 4 days (in a CO₂ incubator at 34°C). MM.1S cells were pre-labeled with 5 μ M cell trace violet (Thermo Fisher Scientific), 10 μ L MM.1S with a cell density of 4 million/ml were seeded into culture chambers (2×10^4 cells/ chamber). The device was then placed inside a CO₂ incubator at 37°C for 4 h to let

MM.1S settle prior to starting the flow. Cells were co-cultured for another 24 h, and then treated with bortezomib for 4 h. After drug treatment, medium was changed and cultures were allowed to recover for a 24 h period before analyses were conducted. MM.1S alone, cultured in the device but under static conditions were used as control.

Cell viability after treatment and recovery was determined as described above, using LIVE/DEAD Viability/Cytotoxicity Kit (0.1 μ M calcein AM & 0.1 μ M EthD-1). Culture chambers were scanned using HCS (CellInsight™ CX5, Thermo Fisher Scientific). Cell trace violet⁺ calcein AM⁺ cells were identified as live MM.1S and cell trace violet⁺ EthD-1⁺ cells were identified as dead cells, using pre-design CX5 software algorithms (HCS Studio 2.0 Cell Analysis Software) for cell quantification and rapid analysis.

Fluorescent images captured by HCS are shown in Fig. 8a. Due to the lower shear stress and auto-fluorescence on the edge of the culture chambers, we scanned and analyzed the 9 fields in the middle of each chamber. The clearness of the images indicates that the polyester membrane allows efficient exchange of liquid and enables proper staining and identification of cells despite it being auto-fluorescent in the green (excitation/emission (nm): 485/521) and red channel (excitation/emission (nm): 560/607). This artefact was eliminated during image process and analysis to ensure proper counting of live/dead cells. Importantly, the membrane prevented floating or loosely adhered cells from being removed during the staining procedure - a design advantage that is critical to retain and count both suspension and dead cells.

Furthermore, images of MM cells alone show an even distribution of cells throughout the well, indicating that nearly no flow disturbance was generated during cell washing and staining. Of note, bubbles in the culture chamber could be removed automatically through the membrane within a minute. Bubbles in the channels could be also easily pipette into culture chamber or directly pipette out with a hard force.

Data quantification was performed automatically using the HCS Studio Software. The percentage of live (cell trace violet⁺calcein AM⁺ cells/cell trace violet⁺ cells) and dead (cell trace violet⁺EthD-1⁺/cell trace violet⁺ cells) MM.1S cells were plotted against bortezomib concentration. MM.1S in co-culture (Figs. 8b and 8c) remained significantly more viable (~70%, $P < 0.05$) between the treatment range of 0 nM to 10 nM compared to cells cultured alone (viability ~60% at 5 nM and ~15% at 10 nM), suggesting that MM.1S cells can develop drug resistance when co-culture with OSB.

Statistics

Statistical analyses were conducted using GraphPad Prism V.7.0. Differences between two groups were calculated using Student's t-test. One way ANOVA followed by Tukey's multiple comparison analysis was conducted to compare multiple groups. A $P < 0.05$ was considered significant.

Discussion

The gravity-driven flow strategy has been previously used to develop pumpless microfluidic culture devices. In comparison to other such systems,^{19,30–32} our design enables uniquely: (1) the control and maintenance of medium flow within 10% of the set rate (Figs. 4 and 6) whereas 50 % was typically achieved by the others;³⁰ (2) the placement of the flow rate-controlling membrane in the inlet port (Fig. 2), instead of embedding it in microchannels which can invite difficulties in device fabrication; and (3) the simple use of a programmable rocking platform (Fig. 1a) in comparison to other gravity-generation methods such as horizontal reservoirs³³ and droplet dispenser.³⁴

Also, for the first time to our best knowledge, we used a transparent porous membrane at the top of the cell culture chamber to facilitate high-throughput *in situ* cell staining and imaging (Fig. 2). Conventional cell staining procedures in other microfluidic devices involve pipetting the staining solutions into the cell chamber through channels, which generate a large shear flow. As a result, cell morphology can change and non-adherent as well as less viable cells could be detached and flushed out. Furthermore, staining solutions are passively introduced into the culture chambers, drastically decreasing the staining efficiency.³⁵ In our design, the membrane use enabled the retention of loosely-adherent cells in the chamber while avoid bubble formation.

The high-throughput capacity of the pumpless culture platform was demonstrated in this study through the evaluation of MM.1S cells' drug response using HCS in three different aspects: (1) 20 culture chambers in a single well plate, (2) dynamic culture without the setting up difficulties and time required to use tubing and pumps, and (3) *in situ* staining and HCS. In regard to the use of *in situ* staining and HSC, entire end-point viability characterization procedures on 20 chambers took less than 1 h. In comparison, conventional characterization based on flow cytometry typically consume over several hours to harvest, stain, and analyze cells.

Our culture platform has also been optimized for: (1) device fabrication based on rapid prototyping using digital cutter, the whole fabrication process takes only 2 hours and (2) device sealing by incorporating a PDMS layer. We developed a method to firmly adhere the PDMS to PSA double-sided tape, which also provides a convenient method to indirectly adhere PDMS to polystyrene surface. We observed that the PSA layers did not cause leaking and bubble problems and adversely affect cell viability during culture. These observations are consistent with results from other microfluidic devices fabricated using PSA.^{26,36} In the current device design, PDMS is used to fabricate the microfluidic device and in direct contact with cell culture medium. It has been reported that PDMS could leach hydrophobic components from cell culture media and store them with in the bulk structure of PDMS.³⁷ To eliminate PDMS, we will need to find a better material to replace the rigid glass bottom, e.g., tissue culture-treated polystyrene plate, in order to seal the culture chamber. Inlet, outlet and cell seeding ports could also be drilled from polystyrene plate. Furthermore, for high-throughput commercial manufacturing, injection moulding could be applied to fabricate device parts, thermal bonding, solvent bonding or adhesive bonding could then be used for assembly.

The pumpless culture platform stimulated osteoblastic differentiation and enabled the formation of a distinct cellular morphology due to the dynamic culture. This flow-induced shear stress is essential for the long term maintenance of OSB and PMMC¹⁶ and is lacking in other comparable *in vitro* models of MM.^{5–7,9,10,14} Importantly, we demonstrated the compatibility of the system with high-throughput staining and HCS screening for drug evaluation purposes of a complex multicellular environment containing OSB and MM cells. In the future study, we plan to use the device to prospectively correlate the *ex vivo* response of PMMC to drugs and short-term clinical outcomes.

In summary, the developed features could significantly increase the potential of our culture platform to be used for preclinical evaluation of new therapeutics, studying the development of drug-resistant cell subpopulations, and identifying novel therapeutic targets. Furthermore, our platform can be extended to develop *ex vivo* models of breast and prostate cancers that metastasize to the BM and bone.

Conclusions

We developed and demonstrated the 96-well plate based pumpless culture platform for high-throughput drug evaluation of multicellular cultures. The culture platform was designed, fabricated, and evaluated to: (1) control and maintain flow rate within 10% under the gravity-based pumpless configuration, necessary for osteoblastic stimulation while eliminating potential sources for leaking and bubble formation and (2) demonstrate high-throughput *in situ* cell staining and imaging using HCS for drug response evaluation of multicellular cultures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1.

Design strategy for pumpless culture platform: (a) a picture of the 96-well plate-based microfluidic culture device, being rotated by a commercial rocking device, in a conventional incubator; (b) a schematic illustration of medium flow through the culture chamber induced by the hydrostatic pressure difference between the medium reservoirs; (c) a schematic view of the 4 wells used to support 1 culture chamber; and (d) an HCS image showing MM (Red arrows) and OSB cells (white arrows).

Figure 2.

Major design features of the pumpless culture platform: (a) fabricated assembly of microfluidic device components; (b) cell seeding port to direct cell placement into the culture chamber, (c) membrane to control medium flow rate, (d) port surface patterned to mitigate medium evaporation during long-term culture, and (e) culture chamber surface engineered to retain cells during *in situ* cell washing and staining.

Figure 3.
Computational simulations: (a) pressure drop contours through fluidic passages and (b) wall shear stress contours in the cell chamber.

Figure 4.

Flow rate control by controlling the tilting angle of the rocking device with respect to time:

(a) tilting angle changes and (b) corresponding flow rate changes.

Figure 5.

Device fabrication: (a) 4-well section illustration of materials patterned and assembled to a bottomless 96-well based well plate, (b) pictures of digitally cut membranes assembled into the device, and (c) bottom of the fabricated device. The patterned PSA and PDMS layers were used to define microfluidic passages and imbed membranes between the bottomless 96-well plate and the glass plate.

Figure 6.

Flow visualization using the fabricated device: (a) illustration of the experimental configuration, (b) polystyrene microbeads floating on the medium surface captured by a microscope, (c) images showing the 20 μm accuracy of determining the vertical position of microbeads by microscopy as a means of measuring h , and (d) comparison to experimentally measured and analytically calculated values of h . Error bars represent the standard deviation of the mean. Experiments were repeated three times.

Figure 7.

Comparison of dynamic and static culture of OSB (hFOB 1.19 cells) in the pumpless culture platform: (a) fluorescence images of cells on day 4 (b) cell viability after 4-day culture, (c) relative ALP activity on day 4. Green = calcein AM (live cells), red = EthD-1 (dead cells), blue = nuclear stain (Hoechst 33324). Bars represent the mean and standard deviation of 4–5 separate wells. Statistical comparisons between perfusion and static cultures were performed using Student's t-test. * $P < 0.05$, ** $P < 0.01$. Scale bar = 100 μm .

Figure 8.

Effect of bortezomib concentration on: (a) HCS images showing the response of MM.1S to bortezomib when cultured alone or in the presence of hFOB 1.19 cells, (b) the percentage of calcein AM⁺ MM.1S (live MM.1S cells), (c) the percentage of EthD-1⁺ MM.1S (dead MM.1S cells). Representative fields are shown, additional information can be found in the supplementary information 3. Data points represent the mean and standard deviation of 4–5 different wells. * P<0.05, **P<0.01, ***P<0.001 compared to MM.1S alone at the respective concentrations. Scale bar =100 μ m.