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A sandwiched microarray platform for benchtop cell-based high throughput screening

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Abstract

The emergence of combinatorial chemistries and the increased discovery of natural compounds have led to the production of expansive libraries of drug candidates and vast numbers of compounds with potentially interesting biological activities. Despite broad interest in high throughput screening (HTS) across varied fields of biological research, there has not been an increase in accessible HTS technologies. Here, we present a simple microarray sandwich system suitable for screening chemical libraries in cell-based assays at the benchtop. The microarray platform delivers chemical compounds to isolated cell cultures by 'sandwiching' chemical-laden arrayed posts with cell-seeded microwells. In this way, an array of sealed cell-based assays was generated without cross-contamination between neighboring assays. After chemical exposure, cell viability was analyzed by fluorescence detection of cell viability indicator assays on a per microwell basis in a standard microarray scanner. We demonstrate the efficacy of the system by generating four hits from toxicology screens towards MCF-7 human breast cancer cells. Three of the hits were identified in a combinatorial screen of a library of natural compounds in combination with verapamil, a P-glycoprotein inhibitor. A fourth hit, 9-methoxy-camptothecin, was identified by screening the natural compound library in the absence of verapamil. The method developed here miniaturizes existing HTS systems and enables the screening of a wide array of individual or combinatorial libraries in a reproducible and scalable manner. We anticipate broad application of such a system as it is amenable to combinatorial drug screening in a simple, robust and portable platform.

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Introduction

High throughput screening (HTS) technologies have successfully identified bioactive compounds, proteins, and small molecules across a broad spectrum of biological fields[1]; however, screening technologies have not kept pace with the expanding number of potential targets. A vast experimental space has been created at the intersection of the potential targets identified in proteomic and genomic studies, and the chemical space defined by combinatorial chemistries[2] and natural compounds.[3] The disparity between potential targets and the current screening capabilities is confounded by the need to study drug-drug interactions at the early stages of development in order to evaluate potential complications in later-stage trials.[4] Traditionally, HTS is carried-out in dedicated facilities, and access is often limited by high capital costs for automated liquid handling and microscopy. As interest in HTS from varied fields of biological research has increased, a need for cost effective and widely accessible HTS systems has followed. Microscale technologies can help fill this gap in technology by creating simple HTS devices that can be easily fabricated and operated in a scalable manner. Benchtop devices will help decentralize HTS by transferring experimental capabilities from centralized locations to various laboratories or field-testing facilities.

Replicating HTS at the microscale requires isolated reaction chambers for cell-based assays, rapid processing of experiments, high array density to accommodate large chemical libraries, and compatibility with diverse types of chemicals as well as the ability to rapidly test various concentrations and combinations of chemicals. Such devices must be simple to use and inexpensive. Additionally, the ability to easily create and screen combinatorial libraries is necessary for generating high quality hits at the benchtop. Combinatorial screening, and the ability to screen for drug-drug interactions can be used as a means of addressing the potential risks associated with absorption, distribution, metabolism, excretion, and toxicology (ADMET) at early stages of drug development.[5,6]

A number of microscale screening technologies have been developed in attempts to address the technology gap in HTS. For example, live cell microarrays have been developed for small molecule and siRNA screening[7,8], cell hydrogel microarrays have been developed for screening cytotoxicity to metabolic products [9,10] and cell-microenvironment interactions[11], and well-less technologies have been developed for high throughput compound screening[12]. These microarray systems begin to address many aspects of miniaturized HTS; however, they have not been developed as generalized platforms for combinatorial screening, and in some cases, the fabrication of arrayed chemical libraries was complicated. With 1536-well plates experiments have been able to reduce screening costs by reducing reactant volumes and processing step, but this plate-based format is still reliant on liquid and plate handling robotics. Here, we build on existing microscale technologies including toxicology assays [13,14] and microarrays [15,16] to design a generalized microarray platform for benchtop HTS. Furthermore, we aim to design a platform in which arrayed chemical libraries can be fabricated and stored separately from the assay experiments, so that a pre-fabricated arrayed chemical library can be printed at a central facility and used at a remote location.

We present a HTS device that can be operated at the benchtop to screen chemical libraries and combinatorial chemical libraries in cell-based assays. The microscale device is a sandwiched structure of cell-seeded microwells and a matching array of microscale posts. When sandwiched together the arrayed posts are design to address single microwells and create sealed chambers in which a screening reaction can be carried out. With the sandwich microarray we screened a library of 320 natural compounds for potential anti-tumour agents by determining the cytotoxicity of each compound towards MCF-7 human breast cancer cells. In a second screen, we simultaneously delivered the P-glycoprotein (P-gp) inhibitor,

verapamil, in combination with the library of natural compounds to screen for potentiated cytotoxicity with inhibited P-gp function. P-gp is a membrane-bound ATP-binding cassette (ABC) transport protein, and shows a significant role in drug-drug interactions by acting as an efflux carrier, a known mechanism of multi-drug resistance[17]. The microarray sandwich system is designed to process more than 2000 individual assays per slide each assay requiring less than 50 femtomoles of screening compound, and by using standard glass slide geometries the system is designed to integrated into benchtop systems.

Materials and Methods

Fabrication of arrayed microscale posts

Arrayed poly(dimethylsiloxane) (PDMS) posts were fabricated by curing a 10:1 mixture of silicone elastomer base solution and curing agent (Sylgard 184; Dow Corning Corporation) on a silicon negative template. The PDMS elastomer solution was degassed for 15 minutes in a vacuum chamber and cured at 70 °C for 2h before the PDMS moulds were peeled from the silicon masters. The generated PDMS replicas had patterns corresponding to the silicon master with protruding columns and were subsequently used for moulding of PEG microwells. A negative template of arrayed posts (400 μm diameter, 150 μm deep, 600 μm pitch) was created on a silicon wafer, using standard photolithography techniques[18], and the pattern and depth were analyzed with a Dektak surface profiler (Veeco Instruments, Santa Barbara, CA). Photomasks were designed using CleWin Version 2.8 (WieWeb Software, Hengelo, Netherlands) and printed on Mylar™ films at Fineline Imaging, Inc. (Colorado Springs, CO) with 20,230 dpi resolution.

Microwell fabrication

Microwells were micromolding from polyethylene glycol diacrylate (1:1 mixture of PEG 258 and PEG 400; Sigma-Aldrich Co., St. Louis, MO) with 1% (w/w) of photoinitiator 2-hydroxy-2-methyl propiophenone (Sigma-Aldrich Co., St. Louis, MO). Arrays of microwells were bonded to 3-(trimethoxysilyl) propylmethacrylate (TMSPMA) (Sigma-Aldrich Co., St. Louis, MO) modified glass slides. A PDMS stamp of arrayed microscale posts was placed on an evenly distributed film of PEG prepolymer solution on 3-(trimethoxysilyl) propylmethacrylate (TMSPMA) modified glass slide and photocrosslinked by UV light (350-500 nm) for 600 s at 100mW/cm^2 (OmniCure Series 2000, EXFO, Mississauga, Canada). Prior to separation of the PDMS mould and microwells, the PDMS mould was subject to plasma treatment and a glass slide was bonded to the top. The top and bottom glass slides were aligned and bonded by using an alignment block (Supporting Information, SFig. 1) that created a sandwich structure from top to bottom of, a) a glass slide, b) arrayed PDMS posts, c) arrayed PEG-microwells bonded to, and d) a TMSPMA treated glass slide. In this way, the patterns of the top and bottom of the device were aligned and calibrated to the orientation of the glass slides. After removal of the PDMS top from the PEG microwells, the array patterns can be easily re-aligned by making flush the edges of the two glass slides.

Device alignment

To align and sandwich the arrayed PDMS posts and PEG microwells, cell-laden microwells were first removed from cell culture media and placed on a flat, sterile surface. Fresh cell culture media was added to the microwells and excess media was subsequently removed by wiping with a sterile cover slip prior to sandwiching. Using this method ensured that each microwell was filled with culture media prior to drug delivery. Secondly, the chemical-laden post were lower from above and sandwiched to the microwells with gentle, even pressure. Correct alignment of the array posts and microwells was facilitated by use the of alignment features at each end of the device as well as by using a right angle block (SFig. 1) to secure

and align the top and bottom of the device in the x and y planes. As the arrayed posts and microwells were oriented with respect to one another during fabrication, correct alignment of the device was easily attained without magnification in approximately one minute. Additionally, as the top is lowered the edges of the alignment features, at both ends, are matched with the edges of the alignment features on the bottom. To ensure proper sealing during drug delivery and exposure, a small weight was applied to the top of the device (5 glass slides, approximately 10 grams).

Microarray printing

A non-contact piezo-microarrayer (Piezorrayer, PerkinElmer) was used to deposit 2 nL of reagents on array PDMS posts. All printing was performed at 11 °C and 40% humidity. After printing, the chemical chip was kept in humidified condition until use.

Chemical library

A library of 320 natural compounds was a kind gift from M.Glicksman (Harvard NeuroDiscovery Center). The quality of the compounds was assured by the vendor (SPEC, Netherlands) as greater than 80 % pure. Compounds were stored at −80 °C until use. Prior to printing, compounds were diluted to a concentration of 16.7 μM in 1% DMSO in phosphate buffered saline (PBS). Compounds B010, M017, L008, P013, A005 and P011 were purchased separately from SPECS Co. Ltd.

Cell culture

MCF-7 human breast cancer cells (American Type Culture Collection) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum in a humidified 5% CO_2 incubator (ThermoForma Electron) at 37°C. Microwells were seeded by pipetting 1mL of media containing 200,000 cells on to the arrayed microwells. Cells were allowed to settle into the microwells for 30 minutes. Undocked cells were washed with excess media. Cell-seeded microwells were cultured for 12 hours prior to use in the device.

Cell viability analysis

To evaluate the cell viability, cells were incubated with 2μM calcein AM (Invitrogen) in PBS for 10 min at 37°C. Live cells became fluorescent under blue excitation due to enzymatic conversion of the non-fluorescent calcein to fluorescent calcein AM. Dead cells were fluorescent under green excitation after binding of ethidium homodimer to the DNA of membrane-compromised cells. Fluorescent cells were visualized with appropriate filters under an inverted microscope (Nikon Eclipse TE2000-U).

For HTS calcein AM stained microwell arrays were imaged with a GenePix 4100a microarray scanner (Axon Instruments, Union City, CA), and fluorescence images were analyzed with GENEPIX PRO (Axon). To account for variability between independent array experiments a global normalization strategy was implemented. Viability index (VI) was used to evaluate cell viability and calculated as follows:

$$\text{Viability index (VI)} = (\text{Xi} - \mu) / \delta \qquad \text{(Eqn. 1)}$$

Where X_i= live cell fluorescence, μ was the average of all the X_i for all spots on each slide, and δ was the standard deviation of all the X_i for all microwells on each array.

In 96 well plate experiments, cytotoxicity was determined by Alamar blue (Invitrogen), IC_{50} (concentrations at which 50% inhibition of growth) was calculated by Origin 8.0 software.

Scanning electron microscopy

Arrayed posts and microwells were imaged using a FESEM Ultra 55 (Zeiss, Germany) scanning electron microscope. Samples were mounted onto aluminium stages, sputter-coated with Pt/Pd to a thickness of 200 Å and analyzed at a working distance of 20 mm.

Results and Discussion

Device fabrication, operation and characterization

A microarray sandwich system was fabricated from cell-seeded poly(ethylene glycol) (PEG) microwells and an array of polydimethylsiloxane (PDMS) posts (Figure 1A). The top, arrayed microscale posts, were fabricated from PDMS to allow for oxygen diffusion into sealed microwells. A number of biomaterials were used to fabricate the microwell arrays, but many of these materials failed to produce stable microwells. We selected a 1:1 ratio of PEG-diacrylate with MW of 258 and 575 as this prepolymer solution produced microwells that were stable in culture media for 2 or more days and had no discernable effect on cell viability. Our proof of concept design utilized standard glass slide geometries to generate an array of 2100 posts (400 μm in diameter, 150 μm in height; Fig. 1B) matching the layout of arrayed microwells (400 μm in diameter, 150 μm in height; Fig 1C), as the top and bottom of the device, respectively. When aligned and pressed together (sandwiched), each microwell is addressed by a single PDMS post, thus creating an array of sealed chambers each with a volume of 20 nL. A chemical library can be printed on to the ends of the arrayed posts by various printing approaches (this was accomplished in the present work by robotic piezo printing). Multiple compounds can be printed on the same spot by repeat printings on the arrayed posts, i.e. in sequential printings, and subsequently delivered to a microwell to enable combinatorial chemical screening (SFig 2). Compounds printed on the end of posts are transferred to the solution contained in the addressed cell-seeded microwell. In this way, the sandwiched microwells and arrayed posts can be used for cell-based screening of small molecule chemical libraries.

Cells seeded into the microwells adhered to the bottom of the microwells and formed a monolayer (Fig 1E). The number of seeded cells per well can be controlled by the initial cell seeding density to enable the formation of fully or partially confluent cell cultures (Fig. 1F). In the toxicology assays herein, microwells were seeded with 2×10^5 cells per glass slide resulting in 43±7 cells per well. The seeding density was selected so that the density of cells per microwell was similar to that used in a standard 96-well plate assay.[19] Microwells can also be fabricated from cell-repellent polyethylene glycol (PEG) to create cell aggregates of controlled sizes and shapes (SFig. 3). Furthermore, cell-laden hydrogels can be integrated into the microwell arrays to enable culturing of cells in 3D. Thus the device can be used to screen the effects of chemicals on cell monolayers, and potentially with cell aggregates and cell-laden hydrogels.

Accurate yet facile alignment of the cell-seeded microwells and the chemical-laden arrayed posts was possible with the aid of alignment features. Distinct patterns at the corners of the PDMS microwell template produced matching features on both the top and bottom chips for easy manual alignment without magnification. To align and assemble a device, cell-laden microwells were placed on a flat, sterile surface and chemical-laden arrayed posts were lowered from above and sandwiched to the microwells with gentle, even pressure. Alignment was aided by the use of a right angle block as described in the Materials and Methods. By bringing together the two chips, all posts and microwells were sealed simultaneously. Each microwell array was made by moulding against the same arrayed PDMS posts, which was used both as template for microwells and for chemical delivery. The matching of arrayed PDMS posts to microwells eased the alignment process, as any

imperfections produced during fabrication were identical in both halves of the device. Additionally, both halves of a device were bonded to glass slides to reduce changes in shape and size of the patterned features (Fig. 1G,H).

Fluorescent dyes were used to enable imaging of a working device. Concentration gradients of FITC-labelled dextran and rhodamine B were printed on the arrayed posts (Fig 2A). As the arrayed posts and microwells were sandwiched together, the printed solutions (Fig. 2C) were transferred to the solution in the microwells (Fig 2B), without cross contamination and smearing (Fig 2D). Also, no diffusion between neighbouring microwells was observed, as none of the microwells was measured to contain both FITC-dextran and rhodamine B.

Although each sealed microwell contained only 20 nL of culture media, cell viability of MCF7 breast cancer cells, as judged by live/dead (calcein AM/ethidium homodimer) staining, was >90% after 24 hours (Fig. 2E,F). A sealed microwell is isolated from fluid exchange with the surrounding media but, as PDMS is permeable to gases[20], oxygen transport to the microwell cultures is not inhibited. Negative and positive controls of 0.1% DSMO and 0.01% TritonX-100, respectively, resulted in a Z-factor > 0.5 when measuring the mean calcein AM fluorescence from arrayed microwells by fluorescent microarray scanner (SFig. 4). The Z-factor is a statistical measure of the suitability of an assay for HTS that accounts for signal range and variation[21] and a Z-factor of 0.5 is the accepted minimum for HTS. Taken together, the positive and negative cell viability controls, and Z-factor analysis demonstrate that the device can be used to evaluate catholicity in response to chemical exposure.

To test the feasibility of the microarray sandwich system for HTS of candidate cancer drugs, MCF-7 breast cancer cells in microwells were exposed to chemicals in sealed microwells for 24 hours, and cultured for an additional 24 hours in fresh media. MCF-7 cells were seeded 12 hours prior to the experiments in microwell arrays and cultured in minimal media supplemented with 10% fetal bovine serum. Negative and positive controls of 0.1% DMSO in PBS and 0.01% TritonX-100 in PBS, respectively were included in each chemical screen, and cell viability was determined by measuring calcein AM fluorescence in a microarray fluorescent scanner. It is noteworthy that a fluorescent microscope can also be used for these screening experiments.

To validate the device, the response of MCF-7 cells to varying doses of doxorubicin, a known chemotherapeutic[22,23], was measured and compared to assays in a 96-well plate format. Concentrations of doxorubicin from 1 nM to 10 μM were simultaneously analyzed in a single device. The dose-response cytotoxicity profile of doxorubicin for MCF-7 cells is shown in Fig. 2G and H. Despite the 10^4-fold miniaturization, the calculated IC_{50} from the microwell toxicology assays of 12±5.4 nM was in agreement with an IC_{50} = 9±0.8 nM as determined in 96-well plate format. These results demonstrate that the lower number of cells and tested chemical did not reduce the predictive response of the microwell array system. Additionally, the evaluation of the IC_{50} of doxorubicin and the delivery of gradients of fluorescent compounds (Fig. 2B) demonstrates that aqueous soluble compounds can be delivered to individual microwells with the sandwich microarray system.

Chemical library screening

To demonstrate the utility of the proposed platform for HTS, we tested the cytotoxicity of a chemical library of 320 natural compounds against breast cancer cells. The library including positive and negative cell viability controls, arrayed in 384-well plates as single compounds (16.7 μM in 1% DMSO), was printed on the posts at a volume of 2 nL. As the posts and the microwells were sandwiched, the printed arrays were diluted at a 1:10 ratio inside the 20 nL microwell to generate a final concentration of 1.67μM (0.1% DMSO). Each compound from

the library was printed on five adjacent posts. Positive controls were printed on five replicate posts at three locations throughout the array, and were used as intra-array controls to evaluate printing consistency across arrayed posts. No significant difference was observed between intra-array controls (SFig. 5) indicating consistent printing and assay conditions within a single device. Negative controls of 0.1% DMSO, were printed on 200 posts. As a second negative control, no compounds were printed on the remaining posts.

Printing the chemical library for five separate devices took approximately 7 hours of printing time using a standard microarrayer. The cytotoxicity assay required 48 hours of culturing time, and data collection required an additional 10–15 minutes per slide. Thus, within a 3-day period (including 2 days of culturing time) 10,500 assays were processed with five separate devices, and up to 2100 assays can be performed with a single device.

Viability index (VI; Eqn. 1), was used to evaluate cell viability, where low VI indicates high cytotoxicity. The index is a global normalization strategy that accounts for the variability between independent sandwich arrays, thus allowing for assay comparison between arrays. The mean VI of the screened library is shown as a colour intensity map in Fig. 3A, where red represents VI < 0, and green represents VI > 0. The mean VI of the positive viability control was −1.01, and there was no significant difference within the intra- and inter-array control (SFig. 5). The mean VI of the negative viability control was 1.01. Two hundred and eighty-five compounds (89% of the library) had a VI less than the negative control (0.1%DMSO), indicating cytotoxic effects in comparison to the negative control. Thirteen compounds (4%) had a VI less than the positive control (0.01% TritonX-100), indicating cytotoxic effects in comparison to the positive control. The remainder of the library was found to be non-toxic to MCF-7 at the assayed concentrations, potentially due to poor solubility in aqueous solution or due to the lack of cytotoxic effects. Compound B010 (C-B010) had the lowest VI, −1.24. Three compounds that span the VI range resultant from the screened library were selected to confirm cytotoxicity in 96-well format (C-P011, C-M017 and C-B010). As expected, C-P011 (VI = 0.22) and C-M017 (VI = 1.35) were non-toxic at concentrations less than or equal to 10 mM. In contrast, the IC_{50} of C-B010 was determined to be 1.07±0.2 μM, indicating its high cytotoxicity.

C-B010, 9-methoxy-camptothecin, is an analogue of camptothecin (CPT), a naturally derived alkoid with anti-tumour efficacy. Camptothecinoids function as DNA topoisomerase inhibitors disrupting normal DNA replication and transcription and leading to cell death[24,25]. Clinical development of CPT was ceased due to adverse side effects; however, the development of synthetic derivatives has led to the use of CPT analogues for cancer treatment[26]. Substitutions to carbon-7, −9 and −10 have been shown to increase anti-tumour efficacy, and in some cases reducing toxic side effects.[27] 9-methoxy-CPT has been shown to have cytotoxic effects on MCF-7 and other cell lines.[28] The identification of 9-methoxy-CPT as a hit compound suggests that the microscale sandwich device is a useful platform for benchtop HTS.

High throughput screening of drug-drug interactions

To assess the utility of the microarray sandwich system for testing drug-drug interactions, we screened a library of natural compounds while simultaneously delivering a known vasodilator and P-gp inhibitor, verapamil. The response of MCF-7 cells to the chemical library in combination with 10 μM verapamil was analyzed (Fig 4). Comparison of the library screened with and without verapamil is shown in Fig 4A as a colour intensity map. The VI of each compound in the absence of verapamil is shown in descending order (Fig 4A, left), and compared to a colour bar indicating the VI in the presence of verapamil (Fig 4A, right). The library screens (with and without verapamil) are also compared as a scatter plot in Fig 4B. VI data points that fall along the x-y line in Fig. 4B indicate no interaction

between verapamil and the screened compound. For example, in the negative control there was no statistical difference in VI of 0.1% DMSO with or without verapamil indicating that 10 μM verapamil has no effect on the VI of MCF-7 cells. Interaction effects between verapamil and a library compound result in a change in VI, and the greater the interaction effects, the greater the distance from the x-y line. Sixty percent of the library was within one standard deviation (±δ) of the mean distance to x-y line, suggesting insignificant drug-drug interactions with verapamil. This is the case for 9-methoxy-CPT (Fig 4B, yellow; VI_{lib} = −1.23, VI_{int} = −0.9). Other CPT derivatives have been shown to be P-gp substrates including irinotecan (CPT-11)[29]; however, the presence of verapamil did not potentiate the cytotoxic effects of 9-methoxy-CPT in MCF-7 cells in the library screen or in 96-well plate assays (Fig. 4B, SFig 6.)

Hits in the interaction screen were defined as compounds that were ≥3δ negative of the x-y line and resulted in a VI less than the negative control (data points shown in red, Fig 4B). Three compounds (<1% of the library), C-L008, C-P013 and C-A005 (Fig 4C, D) met the interaction-hit criteria. The cytotoxicity of each hit in the presence and absence of verapamil was verified in 96-well plate assays (Fig 4E). C-L008 is an analogue of Ovalichalcone, a compound isolated from the seeds of *Milletia ovalifolia* known to have anti-bacterial and anti-fungal activities.[30,31] C-P013 is an analogue of Amromadendrene, an oil extract of *Melaleuca alternifolia* with anti-inflammatory properties.[32,33] C-A005 belongs to triucallane-type triterpenes, a class of compounds that have been widely used as a gastroprotective, hypocholoesterolaemic, and anti-inflammatories. P-gp has broad substrate specificity and it is possible that C-L008, C-P013, and C-A005 are P-gp substrates, thus leading to the increase in cellular concentrations due to the inhibition of efflux by verapamil.[34,35] It is also possible that the cytotoxic effects are potentiated due to other mechanisms for adverse drug-drug interactions.

With a benchtop operated microarray sandwich system we have identified four natural compounds that have the potential for anti-tumour activities. One compound, 9-methoxy-CPT, is an analogue of CPT, a well-known anti-tumour drug. Derivatives of CPT, including topotecan and irinotecan, have FDA approval for treatment of ovarian, colorectal, and lung cancers.[26] The identification of 9-methoxy-CPT as a hit in our system is a strong demonstration of the ability to generate hits at the benchtop.

While simple identification of hits is an important step in the drug discovery process, characterizing potential drug-drug interactions of the identified hits is essential to an efficient discovery process. Later-stage failures in drug testing are often attributed to complications that arise due to drug-drug interactions, and early testing of such interactions can help avoid costly failures. As such, we have designed the microarray HTS system to be easily adaptable to screening drug-drug interactions and combinatorial libraries.

Rapid analysis of the experimental screening outcomes was possible with a microarray scanner. It is also possible to determine screen results with a standard fluorescent microscope with, or without, automated staging. We characterize the microarray sandwich system as ‘benchtop’ as the majority of device components are easily fabricated and operated with equipment that is common in a modern laboratory. Device fabrication requires a UV light source capable of generating a minimum of 0.2 mW cm^{-2}. Access to a clean room facility was required for fabrication of a silicon template, although template fabrication is commercially available. Fabrication of the chemical array is best accomplished with robotic printing or spotting equipment. Such instruments are common to laboratories or academic research departments in fields that would find use for HTS. A key advantage of this platform over traditional 1536, 384, and 96-well plate HTS technologies is that the chemical library microarrays can be prepared beforehand and stored until use. Further

evaluation of the stability of the chemical arrays is required to determine the storage- and shelf-life of the arrays. Additionally, the microarray format uses 50-fold less assay volume in comparison to the miniaturized 1536-well plate format, leading to potential savings in screening costs.[36,37]

In the microarray sandwich system an array of sealed chambers is created in which isolated cell-based assays are performed. Alignment and sandwiching is simple, and assays are initiated simultaneously. Contaminations between neighbouring assays are prevented as each microwell is sealed. With this technique, higher density arrays can be fabricated as the spacing between assays can be significantly reduced. In comparison to 1536 well plates the sandwich microarray system maintains a greater than 20-times higher assay density (~20 wells cm^{-2} in 1536-well plates and 278 microwells per cm^{-2}). In open microarray systems array density is limited by diffusion of analyte from assays to assays. Miniaturization of HTS also eliminates the need for large quantities of screening compounds. Here, only 40 fmoles of each library compound is required for a single assay.

Conclusions

We have developed a simple microarray sandwich system suitable for screening chemical libraries and combinatorial chemical libraries in cell-based assays at the benchtop. An array of sealed chambers is created by ‘sandwiching’ arrayed posted on a microwell array. In cytotoxicity screening for potential anti-tumour agents, four hits were identified from a library of 320 natural compounds with the system. Three of the hits exhibited toxicity to MCF7 breast cancer cells via drug-drug interactions with verapamil and a fourth hit, 9-methoxy-camptothecin, was identified in the absence of verapamil. The benchtop cell-based assay offers opportunities for rapid and inexpensive chemical screening to the common research lab. We anticipate broad application of such a systems as it is amenable to combinatorial drug screening, is simple, scalable and robust.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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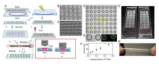

Figure 1.

A microarray sandwich system for high throughput screening. (A) Schematic of the device fabrication and use: a) micromolding of PEGDA microwell array by photocrosslinking; (b) cell seeding in microwell array; (c) a chemical library was printed on to the tips of arrayed PDMS posts by a microarray printer; (d) cell-seeded microwells and chemical-laden arrayed posts were aligned and sandwiched together; (e) after chemical exposure, the cell-seeded microwell array were analyzed for toxicity; (f) a single cell-seeded microwell prior to sandwiching; (g) a sealed microwell assay chamber. SEM images of arrayed (B) PDMS posts and (C) microwells (scale bar = 200 μm). (D) Phase contrast micrograph of a selection of seeded MCF-7 breast cancer tumor cells after 24 hours of culture in a sealed microwell sandwich system. (E) High magnification phase contrast micrograph (left) and fluorescent image (right) of microwells treated with calcein AM (green) and ethidium homodimer (red). (F) The number of cells per microwell as a function of cell seeding density. (G) A photograph of arrayed PDMS posts (left) and a microwell array. (H) A photograph of a sandwiched microarray system.

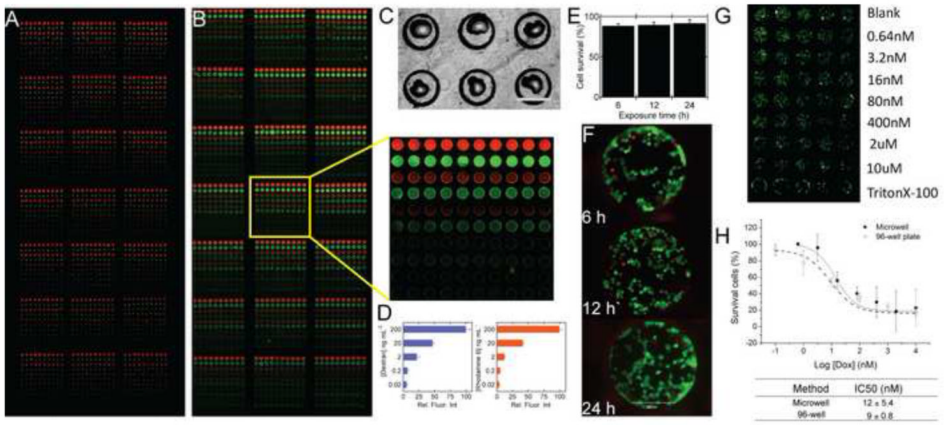

Figure 2.

Characterization and validation of the microarray sandwich system. Fluorescent images of (A) FITC-dextran and rhodamine B printed on arrayed PDMS posts and (B) in arrayed microwells after sandwiching. High magnification image shows a selected 10×10 array of microwells. (C) Micrograph of PDMS posts printed with chemicals (scale bar = 200 μm). (D) Relative intensity of fluorescence of FITC-dextran and rhodamine B from the selected 10×10 array; Ex/Em: 488/525 and 525/550. (E) Cell survival after exposure to PBS in a sealed microwell and (F) fluorescent images of selected wells analyzed with Live/Dead viability assay (green/red). (G) Scanned fluorescent image of a selected array of microwells exposed to various concentrations of doxorubicin for 24 hours and subsequently stained with calcein AM. (H) IC_{50} of doxorubicin as determined in the microarray sandwich system and in 96 well plate format. The lower table compares the values between microwells and standard 96-well plates.

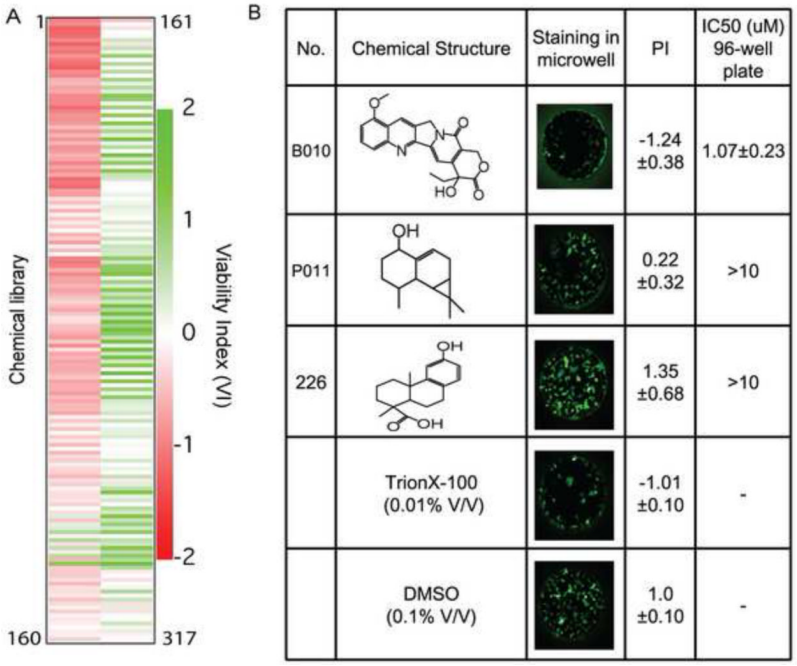

Figure 3.

HTS results of a natural compound library performed in the microarray sandwich system. (A) The mean VI of each library compound shown as a color band. (B) The chemical structures, VI (mean±SE), and IC_{50} of a hit compound (C-B010), two non-toxic compounds (C-P011 and C-M017), 0.01% TritonX-100, and 0.1% DMSO in PBS. C-P011 and C-M017 are nontoxic to MCF-7 cells at the concentration range ≤10 μM.

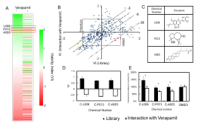

Figure 4.

Benchtop HTS of drug-drug interactions with the microarray sandwich system. (A) Mean VI of the natural compound library in the presence (right) and absence (left) of 10 μM verapamil displayed as color bars. The VI of the library compounds in the absence of verapamil is ordered in descending VI, the VI of each compound in the interaction screen is shown in the adjacent. (B) A scatter plot of the VI of each compound with and without verapamil interactions. Lines indicating the VI of negative (0.1% DMSO) and positive (0.01% TritonX-100) controls are included as visual aids in evaluating the data. (C) The chemical structure of interaction hits, C-L008, C-P013, and C-A005. (D)VI of hits C-L008, C-P013 and C-A005 in the presence (open bars) and absence (solid bars) of 10μM verapamil as measured in the microarray sandwich system. (E) Cell survival relative to negative control for 10μM of C-L008, C-P013 and C-A005 in the presence and absence of 10μM verapamil in 96-well plate.