Quantitative Comparison between Microfluidic and Microtiter Plate Formats for Cell-Based Assays

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Quantitative Comparison between Microfluidic and Microtiter Plate Formats for Cell-Based Assays

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In this paper, we compare a quantitative cell-based assay measuring the intracellular Ca^{2+} response to the agonist uridine 5′-triphosphate in Chinese hamster ovary cells, in both microfluidic and microtiter formats. The study demonstrates that, under appropriate hydrodynamic conditions, there is an excellent agreement between traditional well-plate assays and those obtained on-chip for both suspended immobilized cells and cultured adherent cells. We also demonstrate that the on-chip assay, using adherent cells, provides the possibility of faster screening protocols with the potential for resolving subcelluar information about local Ca^{2+} flux.

The development of robust methods for high-throughput screening (HTS) is important for the rapid assessment of a drug's activity.[1] Traditionally, such measurements, which involve recording the average response of tens of thousands of cells in microtiter plates, have been performed with automated fluid handling robots.[2] Although robotic operation can reduce sample handling errors, effective washing, evaporation, and relative volumetric errors are significant concerns in microtiter plate operation, particularly when the well volume is decreased.[3]

As a consequence, recently there has been considerable interest in trying to translate microtiter-based measurements into microfluidic platforms. Such miniaturized microfluidic systems can provide better precision in generating sample concentrations and improved efficiency in washing.[4] Chip-based assays also use smaller numbers of cells, have a low volume of operation (submicroliter or nanoliter), and have general flexibility in the control of hydrodynamic conditions under which the assay is performed.[5,6] However, the uptake of microfluidics by the pharmaceutical industry in cell screening, and its potential to become a versatile and robust method for pharmacological assessment of new medicines, relies on the ability to generate comparable quantitative information on-chip, with respect to that obtained using current technology (i.e., standard multiwell plates).

Within the pharmaceutical industry, ligand-gated ion channels represent >40% of the targets for drug discovery. Many of these channels are modulated by calcium ions, and as such, the intracellular measurement of Ca^{2+} flux remains one of the preferred ways to determine the activity of new drug candidates.[7] Building upon this established work, we use the activity of the agonist, uridine 5′-triphosphate (UTP), which binds to G-coupled protein receptors and triggers the release of internal Ca^{2+} from a cell's endoplasmic reticulum,[8] as a model assay to quantitatively compare new microfluidic formats with established microtiter plate assay procedures.

To date, there has been limited work on the measurement of intracellular Ca^{2+} in microfluidic systems[9−14] and even fewer fundamental studies that evaluate quantitative on-chip cellular assays. We have previously demonstrated that commonly applied hydrodynamic conditions in microfluidics can activate mechanosensitive ion channels leading to an intracellular Ca^{2+} flux[15] (an observation that has profound implications for assay optimization on chip, as the local hydrodynamic conditions have the potential to induce cell responses that mimic pharmacological effects). In order to demonstrate the broad applicability of chip-based assays as a HTS assay, it is important that equivalent information can be obtained in microfluidic systems.

In this paper, we evaluate functional ligand-based ion channel microfluidic assays using both suspended and adherent cells (cultured on-chip) and under appropriate hydrodynamic conditions compare them with traditional microtiter well-plate assays. We show that measuring Ca^{2+} flux from low numbers of discrete single cells, used in the microchannel format, can provide robust

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pharmacological information, which is in excellent agreement with data obtained from larger populations of cells.

MATERIALS AND METHODS

Cell Culture. The Chinese hamster ovary (CHO)-K1 cell line was from ATCC (the American Type Culture Collection). CHO cells were cultured in 25-cm^2 tissue culture flasks at 37 °C in a humidified atmosphere with 5% CO_2/95% air. The cells were incubated in DMEM/F12 medium (Invitrogen) supplemented with 10% fetal calf serum and 4 mM L-glutamine, were grown to near confluence in the culture flasks, and then were suspended with 0.05% trypsin−EDTA solution. Prior to being introduced into the microfluidic chip or the microtiter plate, the concentration of suspended cells was determined using a hemeocytometer. The viability of cells was determined using 0.4% Trypan Blue solution.

Microfluidic Device Fabrication. We developed a reusable microfluidic device, which comprised a glass substrate that could be sealed against a microfluidic gasket, formed by molding a poly(dimethylsiloxane) (PDMS) elastomer against a silicon master. The substrate could be modified prior to assembly to promote the rapid attachment of a high density of viable cells; see below. Microchannels were 500 μm wide, 140 μm high, and 15 mm long, providing a local environment in which we were able to mitigate against local shear stress, at appropriate flow rates.[15]

Substrate Modification. The substrate was modified prior to assembly with collagen to enhance the immobilization of cells, as described previously.[15] These collagen-modified glass substrates were stored in sterilized foil at 4 °C before being mechanically sealed against a 5-mm PDMS gasket, to form an enclosed microfluidic chip. The gasket was sufficiently thick that the channel geometry did not deform during sealing.

The microfludic devices were connected to a KDS 260 syringe pump (KD Scientific Inc.) using ethylenetetrafluoroethylene polymer tubing with an inner diameter of 250 μm. On−off valves (with appropriate fittings and connectors) obtained from Upchurch Scientific Inc. were used to control flow. Tests were carried out with a colored dye solution to show that the chip did not leak even when using flow rates as high as 400 μL/min.

Well Plate Assay. A traditional measurement of CHO cell's response to UTP in a standard microtiter format was used as a standard reference. Intracellular Ca^{2+} level was quantified using Ca^{2+}-sensitive Fluo-4 AM indicator dye.[16] Cell labeling and washing buffers are described in detail in the Supporting Information. Briefly, 50 μL of a cell suspension (at a concentration of 2×10^6 cells/mL) was seeded per well (~10 000 cells/well) in a 384-well plate and incubated overnight at 37 °C in a humidified atmosphere with 5% CO_2/95% air. After removal of the medium, the adherent cells were washed with the Tyrode buffer and incubated with 50 μL of the Fluo-4 AM labeling buffer for an hour at 37 °C in a humidified atmosphere with 5% CO_2/95% air. The labeling buffer was then replaced with 40 μL of fresh Tyrode buffer to minimize the buffering effect of the dye within the cells. Ten seconds after starting recording the fluorescent intensity of cells, either using fluorescence microscopy or using a fluorometric imaging plate reader (FLIPR), 10 μL of UTP test solution (at a range of concentrations between 10 nM and 10 μM) was added to each well. The induced changes in fluorescence intensity were recorded and calculated to reflect the changes in $[Ca^{2+}]$.[16,17]

On-Chip Assay Using Suspended Cells. On-chip analysis of suspended cells required their loading and immobilization inside microfluidic channels, so that they did not move while tracking rapid changes in intracellular Ca^{2+} flux. The procedure for labeling suspended cells was modified from that used for the well plate assay in order to minimize the adverse effects of long periods of incubation of cells with dye. Suspended cells were centrifuged at 100*g* for 5 min to remove the medium and then washed with Tyrode buffer before being resuspended in the labeling buffer to a concentration of ~2.8×10^6 cells/mL. Following incubation at 37 °C for 10 min, the labeled suspended cells were loaded on-chip at a flow rate of 1 μL/min. Once in the channel, they were left undisturbed for 10 min to allow attachment to the collagen-modified channel surface. Any unattached or loosely attached cells were washed away at a higher flow rate (5 μL/min). UTP agonist test solutions in Tyrode buffer were then introduced into the microfluidic platform at the selected flow rates with UTP concentrations from 10 nM to 10 μM.

On-Chip Assay Using Adherent Cells. The use of adherent cells in microfluidics requires on-chip culture. To avoid bacterial contamination, the valves, connections, and PDMS chips were all sterilized using 70% ethanol and were then dried prior to assembly. A sterilized microfluidic device was constructed using a presterilized collagen-modified substrate, as described previously.[15] The microfluidic channel was first primed with sterilized medium. Freshly harvested cell suspensions at different concentrations were then pumped into the microfluidic chip at 1 μL/min. Once in the microchannel, the cells were left undisturbed for 10 min, before being washed in medium. All operations were performed in a class II biological laminar hood. The cell−chip (as a closed system) was then transferred into an incubator at 37 °C in a humidified atmosphere with 5% CO_2/95% air. The system was continually perfused with medium at a rate of 0.5 μL/min overnight. Once cells were confluent inside the microfluidic device, the medium was replaced with Tyrode buffer at a flow rate of 20 μL/min. Finally, the labeling buffer (as above) was introduced and incubated for 30 min for uniform labeling to occur. After further washing with Tyrode buffer, cells were challenged with UTP concentrations over the same range as above.

Data Acquisition and Analysis. Both transmission and fluorescence images were recorded using a Zeiss Axiovert inverted fluorescence microscope coupled to a monochrome cooled CCD digital camera (Andor iXon^{EM}, Andor Technology.). A filter set, containing an exciter D475/40, an emitter E510, and a beam splitter Dichroic 495, was used for the fluorescence imaging. The intensity of the excitation light was adjusted by a neutral density filter (OD = 1) to minimize photo bleaching of the dye. Real-time fluorescence recordings were made for 3 min at a rate of 10 frames/s using an X40 water immersion lens. The CCD field of view was 200 μm × 200 μm.

In situ fluorescence measurements of the response of adherent cells in the microfluidic device were carried out using a confocal microscope (Zeiss LSM 510) with a X63 oil immersion lens and a 488-nm excitation laser. Real-time recordings of the field of view

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(202 μm × 202 μm) were made at both 4 frames/s and as line scans of a single cell recorded at a rate of 0.0075 s/line.

The fluorescence intensity of whole discrete individual cells was calculated using the Andor iQ1.4 image software. The maximum responding amplitude (simplified as response) was calculated as follows; the cell's response was expressed as $(F_{peak} - F_0)/F_0 \times 100\%$, where F_{peak} is the peak fluorescence intensity and F_0 the baseline. All fluorescence measurements were background subtracted.[16] The baseline fluorescence intensity (F_0) was taken as the average background value over 10 s before applying UTP solutions. All measurements were made in triplicate, and standard errors are given unless noted.

RESULTS AND DISCUSSION

Studies Involving the Comparison of Cell Populations and Single Cells. There has been much debate about the relative merit of the use of single cells, particularly in microfluidic devices, compared with that of population studies in microtiter plate assays when evaluating pharmacological responses.[18] For example, in a typical 384 titer plate, up to 10 000 cells may be used per well, which when incubated overnight will give a confluent layer, as shown in Supporting Information, Figure S1A. Upon the addition of an agonist such as UTP, a proportion of the cells respond, as indicated by an increase in fluorescence intensity (Supporting information Figure S1B). Using a FLIPR, the average response of the whole population in a well is obtained and subsequently used in any data manipulation.[17] In our study, a wide range of UTP concentrations were tested using this procedure in order to obtain a dose−response curve, as shown in Figure 1A. Subsequently, the pEC_{50} ($-\log(EC_{50})$) values were determined using standard sigmoidal curve-fitting methods.[17] A mean value of pEC_{50} from 64 duplicated dose−response experiments (i.e., 16 duplicates on 4 individual plates) was 6.2, which corresponds to a mean EC_{50} value of 0.65 μM. This latter value is the concentration of agonist that provokes a response exactly midway between the baseline and maximum response. In pharmacological studies, it is used as a quantitative measure of the agonist's activity.

The microtiter plate format using a population of cells has obvious limitations, including the lack of information from individual cells and a susceptibility to optical artifacts (due to reagent addition, for example). Moreover, attempts at parallel quantification are limited by the reproducibility and uniformity of the cell monolayer in individual wells. Notably, with other types of cells, i.e., primary cells, a heterogeneous culture is generally produced, and in these circumstances, the formation of uniform cell monolayers remains a significant challenge.

In contrast, the microfluidic approach described here provides the capability to reveal heterogeneous information from individual cells as well as cell ensembles. In order to understand how many single cells were needed to produce a comparable measurement to the traditional microtiter using a cell population assay, we performed a statistical study using the response of individual plated CHO cells within a well plate. Using a fluorescence microscope, the average responses of random selected discrete cells, in groups of 10−125, were recorded from within the microtiter well to obtain the dose−response curves and, subse-

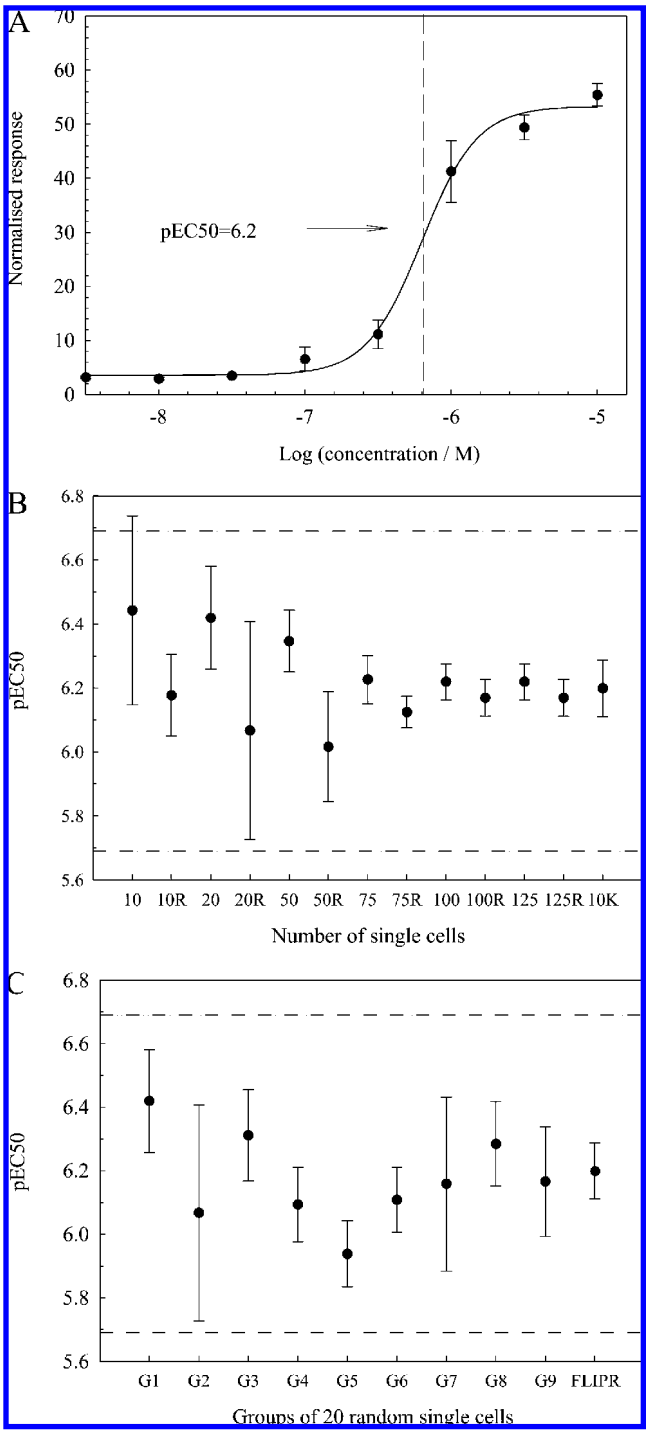

Figure 1. Population response vs single-cell response in quantitative study. (A) A representative dose−response curve from multiwell plates using 10 000 cells/well by FLIPR. pEC_{50} ($-\log(EC_{50})$) wasdetermined by fitting a sigmoid to the concentration−response curve (http://www.graphpad.com/curvefit).[15] Data were normalized to the average maximum value. The mean pEC_{50} from 64 duplicate experiments is 6.20 (±0.03), which corresponds to a mean EC_{50} value of 0.65 μM. (B) Single-cell study using a range of cell numbers. The suffix "R" indicates a duplicate measurement. The acceptable range of the pEC_{50} in industry equals the mean value of pEC_{50} from the FLIPR test 6.2 (±0.5), as indicated by the dotted lines. Any pEC_{50} of a single-cell study with its 95% confidence limit falling into this range was considered reliable. (C) Statistical analysis of a single-cell study using groups of 20 cells randomly selected from within the microtiter plate. Error bar is the 95% confidence limit.

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quently, to determine the pEC_{50} values. The pEC_{50} values were then plotted against the number of individual cells measured and analysized by a Monte Carlo statistical method, as shown in Figure 1B. The mean pEC_{50} value of 6.2 from the population study using 10 000 cells was used as a reference. Any pEC_{50} value whose 95% confidence limit was within the range of 6.2 ± 0.5 was considered reliable.[19] As shown in Figure 2B, all pEC_{50} values obtained from $\geq$20 cells were within the accepted range. An analysis of 9 groups of 20 randomly chosen single cells further demonstrated that all the obtained pEC_{50} values fall within the accepted deviation; see Figure 1C. In summary, the data show that measurements of 20 single cells were sufficient to produce a reliable assay that was comparable to the standard FLIPR measurement of 10 000 cells in the well plate format.

On-Chip Quantification Using Suspended Cells. The introduction of cells into microfluidic devices was generally carried out from a cell suspension, with cells becoming trapped inside a device[20−22] or flowed-through the device for the purpose of handling or detection, i.e., flow cytometry. In most cases, the whole process is rapid, as there is no requirement for culturing the cells. An aim of this work was to assess the potential for using suspended cells (without culture) to give a quantitative Ca^{2+} flux assay on-chip. Due to the rapid intracellular Ca^{2+} flux induced by UTP, it was necessary to immobilize the cells in the microchannel during signal measurement. The microchannel surface was therefore first treated with collagen to achieve high immobilization densities.[15] As shown in Supporting Information Figure S2A, up to 50 single cells (in an area of 0.04 mm^2) could be imaged simultaneously, which allows a random selection of 20 cells for the analysis. UTP concentrations were delivered rapidly and uniformly over cells in the microfluidic channel. At a low flow rate of 2 μL/min (i.e., a fluid velocity of 479 μm/s), it took ~400 ms to exchange the solution in the field of view of the X40 objective. In the standard well plate, the buffer solution is quiescent, and the desired UTP concentration at the cell surface was produced by convective mixing of a small quantity of stock UTP solution within the bulk solution within the well (normally, 50 μL per well for a 384-well plate). In contrast, microfluidic delivery of the drug is both quicker and leads to a more uniform drug distribution across the cell layer. Less than 2 μL of UTP solution is needed to replace the buffer in the microchannel in this study. Supporting Information Figure S2B shows how a cell layer within the microfluidic device responds to 1 μM UTP.

Using the above procedure, quantitative analysis of the response of CHO cells to a wide range of UTP concentrations was assessed on-chip and compared with the 384-well plate. The response of 20 randomly chosen individual cells was measured using fluorescence microscopy. It was known that cells become desensitized after the addition of UTP, and as a consequence, in a microtiter plate it was always necessary to perform parallel assays. Therefore, in order to directly compare data from the traditional microtiter plate with the microfluidic devices, we configured the microchannel as an array of microfluidic structures.

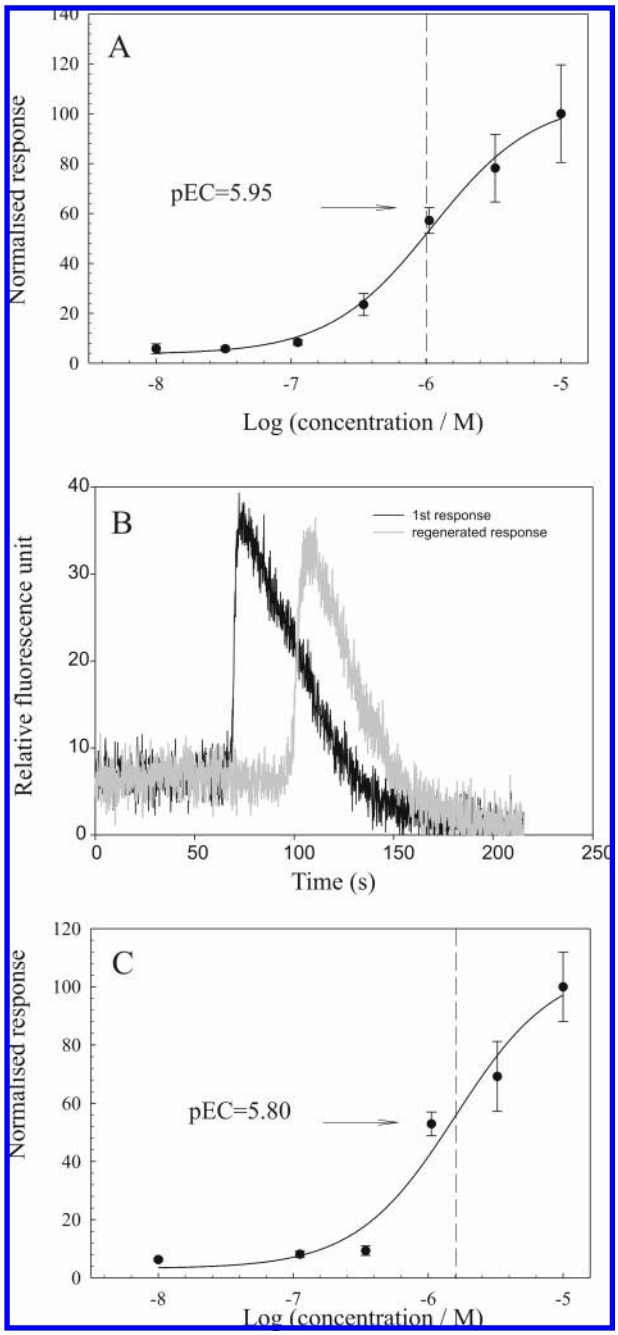

Figure 2. On-chip analysis using suspended cells. (A) A representative concentration−response curve achieved by parallel on-chip analysis at a flow rate of 2 μL/min. The response of ~20 single cells was averaged for a given concentration. The average pEC_{50} obtained from triplicates of on-chip analysis at a flow rate of 2 μL/min is 6.02 ± 0.11, which corresponds to a mean EC_{50} value of 1.0 μM. (B) Sequential on-chip analysis shows that the cell response can be regenerated after buffer washing. In both runs, cells were stimulated with 1 μM UTP. (C) A representative concentration−response curve achieved by sequential on-chip analysis as in (B) at a flow rate of 2 μL/min. The average pEC_{50} value from three duplicate experiments is 5.77 ± 0.10, giving a mean EC_{50} of 1.7 μM. Data were analyzed as in Figure 1. The error bars correspond to the standard error.

Using these devices, we then compared the UTP dose−response curves under a variety of hydrodynamic flow conditions.

Initially, a low flow rate of 2 μL/min (shear stress 0.4 dyn/cm^2) was used to deliver UTP. The dose−response curve generated on-chip showed a sigmoidal relationship, as is commonly

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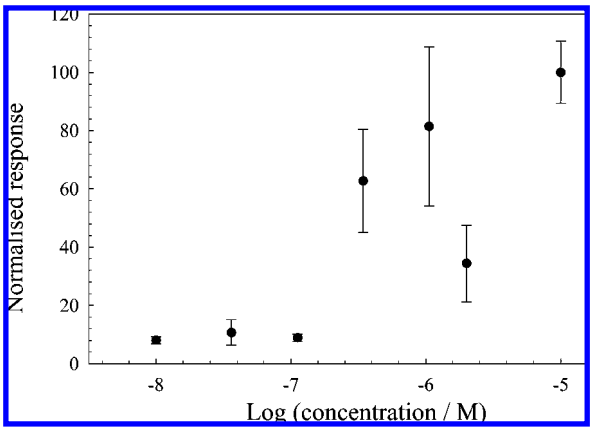

Figure 3. Influence of high shear stress on dose−response curves for suspended cells. A representative concentration−response curve from a parallel on-chip analysis at a flow rate of 20 μL/min. The response of ~20 single cells was averaged for a given concentration. Since a sigmoid curve did not fit the data well, an estimation of pEC_{50} similar to that in Figure 2 was not possible.

obtained in the traditional well-plate assay, Figure 2A. The pEC_{50} value determined on-chip was 6.02 ± 0.11, which corresponds to a mean EC_{50} value of 1.0 μM. To assess the difference between such a parallel on-chip measurement and the standard FLIPR measurement, the *p*-value from a *t*-test was determined to be 0.45. This is much greater than that required for the on-chip mean to be within the 95% confidence limit for the microtiter plate (i.e., *p*-value >0.05).

We also showed that, within the microfluidic format, a rapid exchange of UTP solution can be readily achieved without disturbing the immobilized cells, enabling the sequential testing of the *same* cells. This is of considerable interest for the experimentalist wishing to track the dose−response of an individual cell to a range of agonist concentrations. Again, using a flow rate of 2 μL/min to deliver the UTP solutions, it was found that cells which had become desensitized in a previous test now responded to the same UTP concentration (Figure 2B), after Tyrode buffer washing sequences. The dose−response curve achieved using such sequential UTP/Tyrode washing steps, starting at a low UTP (10 nM) concentrations and increasing to 10 μM, provided similar quantative information (pEC_{50} = 5.77 ± 0.10, corresponding mean EC_{50} = 1.7 μM) to the traditional well-plate assay, Figure 2C. When compared to the standard FLIPR measurement, the *p*-value is 0.09, which again shows that there is no significant difference between the means of the two types of tests.

Importantly, low flow rates were used in the above measurement, since as we have previously shown, shear stress induced by flow rates over a certain threshold (3.1 dyn/cm^2 for the collagen-modified channel) can induce intracellular Ca^{2+} flux.[15] In this context, it is important to note that when UTP solutions are delivered at a flow rate of 20 μL/min (shear stress 4.1 dyn/cm^2), Figure 3, the dose−response curve cannot be fitted using a standard sigmoid relationship. This implies that the hydrodynamic conditions are influencing the cellular response significantly possibly through activation of mechano-sensitive channels.

On-Chip Assays Using Adherent Cells. In contrast to using suspended cells (described above), intra- and intercellular signaling can best be studied using adherent cells, which, when they

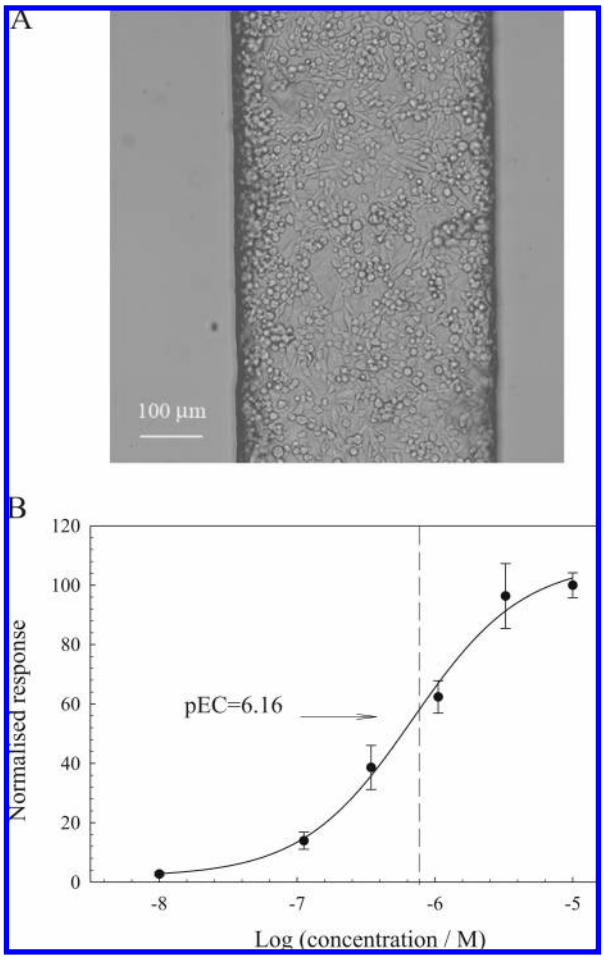

Figure 4. On-chip analysis using adherent cells. (A) A monolayer of adherent cells forms inside the microfluidic channel after 8-h on-chip perfusion at a flow rate of 0.5 μL/min. (B) A representative concentration−response curve achieved by sequential on-chip analysis at a flow rate of 20 μL/min. Data were analyzed as in Figure 2. The average pEC_{50} value from three duplicate experiments is 6.15 ± 0.08, giving a mean EC_{50} of 0.7 μM.

form confluent monolayers, generally give stronger and more uniform signaling. In order to create confluent monolayers of adherent cells inside a microfluidic channel, it was necessary to perform on-chip cell culture, a task that has generally been proven to be difficult as the sterility, the continuous supply of gases, and of medium all need to be controlled carefully.[23] The microfluidic chip developed in this study, however, not only provides a simple way to assemble a sterilized chip but also allows for ready diffusion of gases into the medium, through the PDMS gasket. Low densities of cells (2 × 10^6/mL) were sufficient for seeding the cells inside a collagen-treated microfluidic channel, prior to cell culture. After overnight perfusion with medium at 0.5 μL/min, cells developed into a confluent monolayer with almost 100% viability, Figure 4A.

The effect of shear stress on the on-chip cultured adherent cells was assessed in the same manner as in the study of the suspended cells. No shear-induced Ca^{2+} flux was observed even at high shear stress of 20 dyn/cm^2 (data not shown). In contrast

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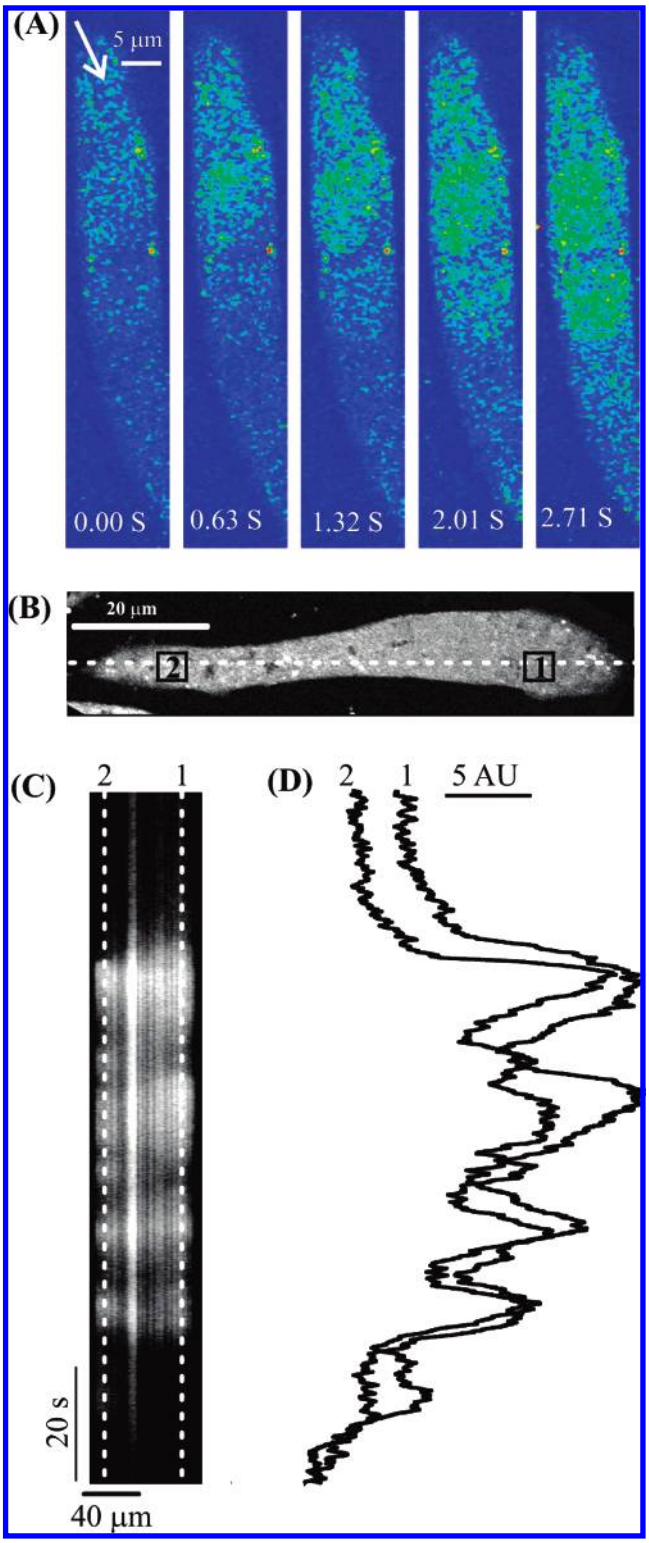

Figure 5. On-chip analysis of intracellular Ca^{2+} wave propagation. (A) Series of confocal images of intracellular Ca^{2+} flux propagation inside a single cell upon delivery of 1 μM UTP solution. The white arrow indicates the flow direction. The Ca^{2+} flux peak travels inside the single cell following the flow direction. (B)-(D) Time course record of Ca^{2+} flux along a fixed line across a single cell upon delivery of 1 μM UTP solution. (B) indicates the line position inside the cell. ROI 1 indicates the front end of the cell to the flow direction; ROI 2 indicates the far end. (C) The 4 distinct fluorescence bands demonstrate Ca^{2+} waves insides the single cell during the 5-min recording. (D) Relative fluorescence profiles from ROIs 1 and 2 show the Ca^{2+} flux arrives at ROI 2 is after that generated at ROI 1.

to the suspended cells, this lower sensitivity to shear stress of the adherent cells can be attributed to their well-formed cytoskeleton, which may serve to reduce the distortion of the cell membrane. With the high tolerance of the adherent cells to shear stress, it was found that substantially higher flow rates could be used for fast cell screening without compromising the cell response. For example, an on-chip sequential assay of the adherent cells in response to UTP was performed in an manner identical to that study using suspended cells (Figure 2C), but at a higher flow rate of 20 μL/min (shear stress of 4 dyn/cm^2). These assay conditions substantially shortened the total assay time, and the pEC_{50} value from measurements performed in triplicate was 6.15 $\pm$ 0.08 (n = 60), Figure 4B. The determined p-value of 0.85 was substantially higher than the p-values from the on-chip assays using suspended cells (0.45 and 0.09), suggesting an excellent comparability between the on-chip assay using adherent cells and the traditional microtiter-plate method.

On-Chip Monitoring Intracellular Signals Using Adherent Cells. Confocal analysis of adherent cells on chip was used to reveal information on the nature of intracellular Ca^{2+} flux. The arrival of a 1 μM UTP dose at 20 μL/min and reaching the cell (arrowed) resulted in a Ca^{2+} wave which "traveled" within a cell in the same direction as the flow rate, Figure 5. Control experiments using Tyrode buffer at the same flow rate induced no Ca^{2+} flux. This phenomenon was further revealed by imaging a line scan across a cell in the direction of UTP delivery (Figure 5B). For the purpose of analysis, a line was constructed between two positions inside the cell, ROI 1 and ROI 2. Four distinct fluorescence peaks were observed along this line, corresponding to a succession of Ca^{2+} waves within the isolated cell, Figure 5C. The relative fluorescence profile from ROI 2 clearly lags behind the one from ROI 1, as shown in Figure 5D. This observation demonstrates the potential of an on-chip assay to resolve local information at a subcellular level, providing a tool for studying the mechanism of signal trafficking and cell pathology. Clearly, the spatial nature of the response would be difficult to observe using a traditional microtiter plate.

CONCLUSIONS

A systematic evaluation of a quantitative functional assay using both suspended CHO cells and on-chip cultured cells in a microfluidic system was investigated. By using intracellular Ca^{2+} analysis of CHO cells as a model system, we found that it was possible to show a close correlation between the suspended CHO cell dose−response for the agonist UTP on-chip and that achieved in a traditional microtiter plate. The use of adherent cells in microfluidics demonstrated a lower susceptibility to the influence of hydrodynamic conditions, providing fast and reliable screening data when compared with that from microtiter plate. The system also demonstrated its potential in controlling and revealing subcellular events.

The outcome of the work quantitatively demonstrated the promise of microfluidics in HTS applications. Information equivalent to that obtained from the microtiter plate has been achieved on-chip with a reduced assay volume and smaller amounts of cells. The precise and multiple delivery of liquid over cells using microfluidics provides additional opportunity to develop new assays, for example, in applications where sequential or dynamic change of cellular microenvironments is difficult to achieve using

microtiter plates. In future, the integration of other microfluidic subunits (such as valves and diluters) or sensors (electrochemical or optical) on-chip may further improve sample handling and analysis, leading to an integrated high-throughput and high-content cell screen.

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SUPPORTING INFORMATION AVAILABLE

Detailed information on buffers for Ca^{2+} flux assay. Description on assembly of microfludic chips. Representative fluorescence images of CHO cell response to UTP in a traditional well-plate assay and in an on-chip assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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