A prototypic microfluidic platform generating stepwise concentration gradients for real-time study of cell apoptosis

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This work describes the development of a prototypic microfluidic platform for the generation of stepwise concentration gradients of drugs. A sensitive apoptotic analysis method is integrated into this microfluidic system for studying apoptosis of HeLa cells under the influence of anticancer drug, etoposide, with various concentrations in parallel; it measures the yellow fluorescent protein/cyan fluorescent protein fluorescence resonance energy transfer (FRET) signal that responds to the activation of caspase-3, an indicator of cell apoptosis. Sets of microfluidic valves on the chip generate stepwise concentration gradient of etoposide in various cell-culture microchambers. The FRET signals from multiple chambers are simultaneously monitored under a fluorescent microscope for long-time observation and the on-chip results are compared with those from 96-well plate study and the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. The microfluidic platform shows several advantages including high-throughput capacity, low drug consumption, and high sensitivity. © 2010 American Institute of Physics. [doi:10.1063/1.3398319]

I. INTRODUCTION

This paper describes the development of a microfluidic device for the generation of stepwise concentration gradients of drugs to simultaneously study cell apoptosis under different drug concentrations. In the past decade, microfluidic technology has shown its great value for chemical analysis and cell biology because of its capability of handling fluids in the microscale and its potential of high-throughput biochemical assays on cells.^{1,2} The advantages of microfluidic devices for the study of cell apoptosis can circumvent the problems encountered by conventional methods and can greatly facilitate the study.³⁻⁷ For example, Valero *et al.*⁴ demonstrated a microfluidic cell trap device to study apoptotic dynamics of HL-60 cells. Tamaki *et al.*⁷ developed a microsystem for cell experiments consisting of a scanning microscope detection system and a cell culture microchip to detect nonfluorescent biological substances with extremely high sensitivity without any labeling materials to monitor cytochrome *c* distribution during apoptosis in a single neuroblastoma-glioma hybrid cell in a glass microchip. Wolbers *et al.*^{5,6} examined autofluorescence intensity to discriminate viable from apoptotic HL-60 cells on a microfluidic chip. Other microfluidic devices have been demonstrated for cell cytotoxicity screening, including cell traps in defined positions⁸⁻¹³ (such as U-shape wires, polydimethylsiloxane (PDMS) wells, C-shaped rings with microsieves, holographic optical traps, and dielectrophoresis), microfluidic drug gradient generator,^{9,14-16} and patterned microsurfaces. Some of these methods rely on the observation of cell morphological changes and proliferations for the determination of cell apoptosis. In addition,

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most of the microfluidic approaches study cell apoptosis under a single drug concentration each time. It is desirable to be able to generate various concentrations of drugs for screening of cell apoptosis on a microfluidic chip. Previously, microfluidic devices have been used to generate concentration gradients in microchannels.^{16–19} For example, by repeating the cycle of combining and splitting solutions of different concentrations, arbitrary concentration gradients can be formed in the microchannel along the direction perpendicular to the flow.^{17,18} These gradients have found many biological applications.^{16,18–20} However, these gradient generators are not favored in some drug screening tests because (1) the formed gradients exist in continuous solution phase (cells may move to different locations during culture by chemotaxis) and (2) in most cases constant flow rate is required. Also, different parts of a cell may experience different drug concentrations in a microchannel having the concentration gradients. A stepwise gradient generator of microfluidics provides a better solution with isolated, miniaturized microchambers that resemble the macroscale well plates for drug screening.

Here we describe a multiple-dilution microfluidic device for the generation of stepwise drug concentration gradients that is used for studying cell apoptotic processes simultaneously under various concentrations. It is believed that the key event of programmed cell death process is the activation of intracellular cysteine-aspartic acid proteases (caspases), which induces the start of cell apoptosis.^{21–24} Nicholson *et al.*²⁵ identified the tetrapeptide of Asp-Glu-Val-Asp (DEVD) as a consensus cleavage site for caspase-3. Based on this discovery, many fluorescent analysis methods are demonstrated to successfully measure caspase-3 activity during cell apoptosis. For example, a cell-based detection system has been developed for monitoring the dynamics of caspase-3 activation in live cells. This system uses a fluorescence resonance energy transfer (FRET) probe generated by fusing a cyan fluorescent protein (CFP) and a yellow fluorescent protein (YFP) with a peptide linker containing the caspase-3 cleavage sequence DEVD.²⁶ Activation of caspase-3 is a hallmark event of apoptosis;^{21–24} during cell apoptosis, activated caspase-3 cleaves the peptide linker to separate YFP from CFP and alters the FRET signal. By measuring the relative intensities of the cyan and yellow fluorescence (i.e., the FRET signal) of HeLa cells transfected with the plasmid of this FRET probe at different time, one can determine whether, when, and how fast the cells go through apoptosis. Using the stable cell line of HeLa-C3 cells which can constitutively produce this caspase sensor, ~3 fold changes in the emission ratio between the acceptor and donor upon the activation of caspase-3 was detected from previous studies.^{26,27} In this study, we introduce this system into a microfluidic chip that can run multiple apoptotic assays of HeLa-C3 cells at various drug concentrations simultaneously. Different from the conventional MTT assay which measures the activity of dehydrogenase enzymes (UV-visible absorption is measured) in cell populations, the FRET system directly analyzes the activity of caspase-3 in same cells at different time points. This system is easier to be integrated into a microfluidic chip than conventional methods. Stepwise gradients of drugs in microchambers are generated on the chip with integrated microfluidic valves to mix drug stock solution with culture medium at different ratios. We monitored real-time signal changes in each individual cell during cell apoptosis under the influence of drugs at various concentrations with fluorescence microscopy. The validity of this microfluidic technique is confirmed by comparing the on-chip results with those from standard MTT assay and 96-well plate assay.

II. EXPERIMENTAL

A. Materials

Negative photoresist SU-8 2050 and positive photoresist AZ 4620 were from MicroChem, Newton, MA and Clariant Corp., respectively. PDMS was purchased from GE Silicones RTV 615, CT. Crystal violet, sodium fluorescein, and sulfuric acid were ordered from Sigma-Aldrich.

B. Cell seeding and culture

The cell line HeLa-C3 was manipulated under sterile tissue culture hoods and maintained in Eagle minimum essential medium (MEM)/10% fetal bovine serum (FBS) supplemented with

100 u/ml penicillin, 100 $\mu\text{g/ml}$ streptomycin (all purchased from Gibco, Invitrogen, NY), 50 $\mu\text{g/ml}$ G-418 solution (Roche, Switzerland). The cells were cultured in humid incubator at 37 °C with 5% CO₂. The device was sterilized under the UV treatment for 30 min before use. The surface of channels was treated with a 0.01% poly-L-lysine (PLL) (Sigma) solution for 30 min to enhance cell adhesion. The cell passage was achieved with standard 0.25% trypsin (Gibco, Invitrogen, NY) and neutralized with MEM medium. The cell suspension solution was centrifuged at 1250 rpm for 3 min and the cells were resuspended in the MEM medium. The cells were counted with a hemocytometer and diluted to the density of 5×10^7 cells/ml. A programmed syringe pump (Harvard Apparatus, MA) injected the cell suspension into the microfluidic device.

C. Drug exposure and analysis

The stock solution of etoposide (Sigma) was 100 μM . After cells were seeded on the chip for 12 h, the drug was perfused into the cell chambers. Multiple concentrations of etoposide were generated in different microchambers on the microfluidic chip for apoptosis study in parallel. Fluorescent images of the HeLa-C3 cells were captured by an inverse fluorescence microscope (Nikon Eclipse TE2000-U, Japan) at different times (0, 12, 24, 36, and 48 h) with excitation filter at 436 ± 20 nm, a dichroic mirror at 455 nm, and emission at 535 ± 15 nm (YFP) and 480 ± 20 nm (CFP). Images were recorded with a computerized cooled charge coupled device camera (Diagnostic Instruments, MI). The cell culture chip was kept in a cell incubator at 37 °C with 5% CO₂ supplement at all times except for imaging.

The fluorescence signals from apoptosis markers of FRET probes (CFP and YFP fusion protein) were analyzed using image processing and analysis software (ALPHA-EASEFC, CA). The fluorescence of each cell was corrected by subtracting the averaged local background.

To verify cell apoptosis, immediately after the on-chip study, 4 $\mu\text{g/ml}$ Hoechst 33342 (Molecular Probes, Eugene, OR) was perfused into the channel and incubated at 37 °C for 20 min. The cells were rinsed with phosphate-buffered saline (PBS) and imaged immediately.

D. Measurement of FRET signals on a 96-well plate

Cells were grown in a 96-well plate overnight with a density of 5×10^3 – 8×10^3 per well and treated with etoposide at different concentrations. At different time points, emission signals of YFP (535 ± 8 nm) and CFP (486 ± 8 nm) were measured by a fluorescent plate reader (Perkin-Elmer Victor, MA) with excitation at 440 ± 10 nm. The background fluorescence was measured from the wells containing only the culture medium.

E. MTT assay

Cells were grown in a 96-well plate and treated with the drug at different concentrations. At the indicated time points, 10 μl of the MTT labeling reagent (Sigma) was added into each well and the plate was incubated for 4 h in a cell incubator before a 100 μl solubilization solution was added into each well. The plate was incubated overnight in the incubator before its absorbance at 595 nm was measured with a plate reader (Perkin-Elmer Victor, MA).

III. RESULTS AND DISCUSSION

A. Microchip fabrication

The microfluidic device was fabricated with standard multilayer soft-lithographic technology with slight changes (Fig. 1).^{28,29} In normal PDMS peristaltic valves,^{28,30} fluid channels are normally low (maximum height of ~ 10 – 20 μm) with a rounded cross section; the maximum height of the channel is limited by the thickness of positive photoresist that can be obtained with spin coating during photolithography. Cells (~ 10 – 20 μm in size) that flow in such low channels are very easy to stick to channel walls and to break. To avoid this problem, we made high microchannels (~ 80 μm high) with a rounded cross-sectional profile by replicating high features of positive photoresist from negative SU 8 photoresist, which is well known for tall features to 1 mm thick.

FIG. 1. Schematic illustration of the fabrication of the microfluidic device.

Silicon masters with SU 8 photoresist are generated with photolithography. The channel design is patterned into SU 8 photoresist of $\sim 130\text{ }\mu\text{m}$ thick, which had a rectangular cross section. A PDMS channel is then molded from this SU 8-silicon master at a $70\text{ }^{\circ}\text{C}$ oven for 1 h. After conformally sealed with a glass slide, the PDMS channel is filled with positive photoresist and placed in a hood overnight to remove the solvent in the photoresist. After the photoresist is dry, the PDMS channel is peeled off to leave a photoresist mold ($\sim 80\text{ }\mu\text{m}$ thick with solvent removed) on the glass. This glass slide is subsequently placed on a $100\text{ }^{\circ}\text{C}$ hotplate for 10 min to melt and round the photoresist pattern. A thin layer of PDMS prepolymer is spin coated onto this rounded photoresist pattern; heating the PDMS prepolymer in a $60\text{ }^{\circ}\text{C}$ oven for ~ 8 min partly cures the PDMS layer.

Another SU 8 master with different design is used to generate open PDMS channels for valve control. The valve control channels are aligned and placed onto the partly cured PDMS layer on the rounded photoresist pattern; heating in a $70\text{ }^{\circ}\text{C}$ oven fully cures the PDMS and bonds the two PDMS layers together. After plasma treated for 1 min, this multilayer PDMS piece is permanently bonded with a flat PDMS substrate to form the entire PDMS device. The control channels are filled with water and controlled by screw-driven structures,³⁰ which make the setup convenient to place the whole setup in cell incubator.

B. Generation of stepwise gradients of drug concentration on microfluidic chip

One major advantage gained by using microfluidic chips is that multiple samples can be studied simultaneously, thus increasing the throughput and reducing the error by running the samples in parallel. Figure 2 shows the design of the microfluidic device that is used to form stepwise concentration gradients of compounds in parallel. It can be conveniently fabricated by soft lithography and has two layers. The bottom layer is the fluidic channel network in a “ladder” shape with four fluid inlets/outlets. The top layer is a system of three groups (groups 1–3) of microvalves for controlling the fluid flow underneath; each group consists of many microfluidic valves that are connected to each other and synchronized in operation. Valve group 2 blocks all the middle channels at the rung position of the ladder in the fluidic layer and forms one microchamber (middle chamber) in each “rung” channel. When closed, valve group 2 and group 3 form two side microchambers (side chambers) in each rung channel. The position of each valve of group 3 is shifted from its neighbors so that the volume ratio of solutions at the two side chambers is changed along the ladder. If the solution at one side is buffer for dilution, stepwise gradient of solution concentration can be created in the microchambers along the ladder. The exact concentration in each microchamber is determined by the original concentration of the solution and the volume ratio of the side chambers. We achieved two orders of magnitude of dilution in our current device. In principle, more dilutions can be obtained. Valve group 1 is used to close the side channels to ensure that an injected solution, particularly cell culture, will flow through the rung channels without any bubble trapped; the trapped bubbles make it difficult to seed cells in the middle chamber (see section below).

We demonstrate the capability of this device for the generation of stepwise concentration gradients by mixing an acid solution and de-ionized (DI) water at increasing ratios to create solutions with increased pH values in the chambers. Figure 2(a) is the illustration of the process with corresponding images of the microchip in Fig. 2(b). After valve group 1 is closed, a 5% crystal violet solution (a pH indicator) is filled into the entire channel. Valve group 2 is closed to trap a small amount of the solution in the middle microchambers and segregate the flows in the channels at the two sides of the ladder, which are filled with an acid solution (0.5 M sulfuric acid) and DI water, respectively. Valve group 3 is closed to accurately meter an intended amount of acid solution and water in the side chambers. Valve group 2 is opened to allow the acid and water to mix and generate stepwise pH gradients in the microchambers along the ladder. The pH change in different microchambers can be clearly seen from the color variation in the pH indicator.

FIG. 2. Operation of the microchip to generate stepwise gradient. (a) Schematic illustration of the operation. Valve channels (groups 1–3) are shown in red (valve is closed) and pink (valve is open). The fluidic channels are in the shape of a ladder (blue, gray, and black colors stand for different solutions). [(b)–(d)] Generation of stepwise gradient of pH in the microchambers. Crystal violet (a pH indicator) is added into the solution to visualize the formed pH values. The lower three figures in (a) correspond to (b), (c), and left half of (d), respectively.

C. Quantification of solution concentrations in the microchambers

We quantify the concentrations of solutions formed in the microchambers with sodium fluorescein. Fluorescein solutions with different concentrations (0–10 mM) are injected into the microchannels and fluorescent intensity in the microchambers is measured. All the fluorescent intensities are offset with the fluorescent intensity of pure water (0 mM fluorescein). Within the measured concentration region, fluorescent intensity increases linearly with concentration [Fig. 3(a)].

With the operation in Fig. 2, stepwise gradient of fluorescein solution is generated in the microchambers. The concentration of solution in each chamber can be calculated by multiplying the original solution with the dilution factor, which is the ratio of the channel volumes between valve 2 and valve 3. This calculated value of each chamber is compared with the corresponding measured value [Fig. 3(b)]. The two values match very well with each other. For convenience, we directly use the calculated concentrations in the microchambers in subsequent experiments.

D. Cell seeding and culture on chip

The same chip design is used for drug screening of cytotoxicity (only apoptosis is monitored). Cells are loaded into the microfluidic chambers by following an adapted procedure.

FIG. 3. Quantification of fluorescein concentrations in the microfluidic chambers. (a) Fluorescent intensity of fluorescein solution in the microchamber changes linearly with its concentration. (b) Comparison of measured fluorescein concentrations in the microchambers and calculated values of concentrations. The calculated values are obtained by multiplying the original fluorescein solution concentration with dilute factors, which are determined by the chip design.

1. Surface treatment

To enhance cell attachment and spreading on PDMS surface, PDMS pieces are oxidized in oxygen plasma before they are sealed to form microfluidic channels. The surface of the sealed microchannels is immediately treated in a 0.01% PLL solution for 30 min. The positively-charged PLL polymers adsorb to the negatively charged PDMS surface by electrostatic force. PLL treatment has been demonstrated previously to be able to enhance cell attachment onto surfaces. Our results show that all HeLa-C3 cells can attach to the PLL-treated hydrophilic PDMS substrate within 2 h after seeding and spread well within 4 h. We found that for cell attachment and spreading, the PLL-coated PDMS substrate is comparable to the commercial polystyrene culture dishes with tissue culture treatment.

2. Load cells

Valve group 1 is closed to force the cells to flow through the rung channels. After cells are distributed in all the rung channels, the flow is stopped and cells are allowed to settle and attach to the bottom surface of the PDMS channels.

3. Trap cells

Valve group 2 is closed to form isolated microfluidic chambers. During the cell loading step, cells are seeded in both the rung channels and the side channels. Because the size of each cell is about 1/20 of the channel size, the cells that attach on the side channels need to be removed to meter solutions accurately in the next step. With valve group 2 closed, sterilized DI water is flowed into the side channels of the system until all the cells outside are swelled and broken because of osmotic pressure. The cells in the middle chambers are not influenced with valve group 2 closed.

4. Meter solutions and generate stepwise gradients

Culture medium and drug-containing culture medium are injected into the two side channels, respectively. Valve group 3 is closed to form side chambers that trap the two solutions with accurate volume. Finally, the valve group 2 is opened to allow the solutions from the two side chambers to mix; because the volume ratios of solutions at the two side microchambers are different in each rung channel, stepwise gradient of drug concentration is created along the system.

5. Cell culture

The whole chip is placed into a cell incubator at 37 °C for cell culture. The use of a screw-driven microvalve design that we developed previously²⁴ avoids complicated connections of bulky external pressure source; the entire microfluidic device can be placed into a conventional cell incubator for the optimum control over both temperature and CO₂ concentration (PDMS is gas permeable).

The side channels are $\sim 1\text{--}1.5$ mm long and the microchamber size is $300 \times 500 \mu\text{m}^2$. The diffusion constant of small molecules in water at 37°C is in the order of $10^{-5} \text{ cm}^2/\text{s}$. Estimated by the diffusion equation $x^2 = 2Dt$, where x is the diffusion distance within time t and D is the diffusion coefficient, it takes about 20 min for the drug molecules to diffuse over this distance and form homogeneous solutions in each microchamber. This time can be reduced with short side channels. Compared with the time required to induce cell apoptosis with etoposide, which is normally over 10 h,^{27,31} this time for mixing is short and negligible.

The medium used on the microchip is supplied with 15% FBS. For screening assays, it is important that the nutrient in the enclosed environment (both the middle microchamber and the side channels) is sufficient to support the cell growth. On average, each microchamber is loaded with around 20 HeLa-C3 cells. The total volume of the enclosed space is tens of nanoliters. We found that this volume can sustain cells to live healthy for at least 2 days. If needed, fresh culture medium can be changed by repeating the procedure above.

E. Single-cell apoptosis assay under drugs with stepwise concentration gradients

To demonstrate the capability of our system for real-time study of cell apoptosis at the single cell level, we chose etoposide, a commercial anticancer drug commonly used in chemotherapy, as cell apoptosis inducer on HeLa-C3 cells. The stepwise gradients between $0\text{--}100 \mu\text{M}$ are generated with the device shown above. The moment when the drug solution starts to be diluted is set as the zero time point. Phase contrast image, YFP and CFP fluorescence images of each cell in all microchambers are recorded every 12 h for 2 days. The ratio of YFP and CFP fluorescence intensities of each cell is used as the indicator of cell apoptosis. YFP and CFP ratios of each cell at different times are normalized by setting the ratio of the cell at the zero time point as 1.

In order to quantitatively define the inducement of the apoptosis in gradients, we could detect the activation of caspase-3 inside each living cell by calculating the relative emission ratio of YFP/CFP in the fluorescence images and determine the temporal relationship between caspase-3 and the apoptotic event. Before caspase-3 is activated, the cells are in normal cell shape and the fluorescent light is emitted primarily from the YFP. When caspase-3 is activated during drug-induced apoptosis, the sensor C3 is cleaved and the fluorescent light is emitted predominantly from the CFP (Fig. 4).

Figure 5 shows typical FRET results of five individual cells at different drug concentrations. In general, the YFP fluorescence decreases while CFP fluorescence increases with time. The relative YFP/CFP ratio begins to decrease gradually, and a fast drop of this ratio indicates the irreversibility of cell apoptosis. Individual cells show significantly different profiles with different apoptosis-initiation times even under the same conditions. The result suggests that individual cells can have different sensitivity to the same drug. We found that at low drug concentrations a few cells (less than 10%) show distinct profiles where the relative YFP/CFP ratio increases slightly at the beginning of the experiment. The exact reason needs further investigations.

Cell apoptosis is confirmed by two other commonly used methods. The first one is to monitor the change in cell morphology, which can indicate cell apoptosis. From the phase contrast images, cell morphology changes a little within the first 12 h at all concentrations of etoposide. With longer treatment time, the cell shape becomes abnormal with cells starting to shrink. The other method is to observe chromatin condensation and fragmentation, which are the symbols of apoptosis. Hoechst, a cell permeable fluorescent nucleic acid dye, is applied to the cells to reveal these phenomena by staining the condensed nuclei of apoptotic cells. In Fig. 6, the cells with shrunk cell morphology displayed chromatin condensation and fragmentation, while others with normal cell shape appeared with much weaker Hoechst staining. The results of both methods are consistent with the FRET analysis of cell apoptosis.

To check the quality of the culture medium in the chamber, we set a control group that has no anticancer drug in the MEM 15% FBS medium for 48 h. By analyzing the relative YFP/CFP ratio, it is evident that the nutrient is sufficient for the cells (~ 20 cells/chamber) and the caspase-3 is not activated under that condition (Fig. 7).

FIG. 4. Representative phase contrast and fluorescent images of HeLa-C3 cells in a typical microchamber treated with etoposide ($37 \mu\text{M}$) at different time points. [(A)–(E)], [(a)–(e)], and [(α)–(ϵ)] show the phase contrast, YFP fluorescent, and CFP fluorescent images, respectively. The line-shaped shadow in the phase contrast images is from the diffraction of light at two sides of the microchamber, which are curved under the influence of the valve control channels.

F. Comparing on-chip apoptosis assay with conventional assays

The single-cell results are averaged from over ~ 100 cells and compared with two other assay methods (Figs. 5 and 6). In the first method, cell apoptosis is studied in a 96-well plate with each well containing 5000–7000 of HeLa-C3 cells [Fig. 8(a)]. The results in the relative emission ratio of YFP/CFP show the same trend as those obtained from the microchip. However, the FRET signal obtained from a large number of cells grown in the 96-well plate with the data obtained from a plate reader is not as strong as the data obtained from a small number of cells during the on-chip study. The YFP/CFP emission ratio of the plate system changes approximately 2–3 folds in 2 days after the drug addition, while from our chip-based single cell analysis, the ratio is around fivefold. The major reason comes from the difference of optical filter systems of the fluorescent microscope and the plate reader we have. The emission peaks of YFP and CFP are 526 and 480 nm, respectively. The emission filters in the microscope for YFP and CFP are 535 ± 15 and 480 ± 20 nm, respectively; both cover well the emission peaks of YFP and CFP. However, the corresponding plate reader's filters are 535 ± 8 and 486 ± 8 nm, which barely cover the emission

FIG. 5. Single-cell apoptosis analysis. [(a)–(c)] show the monitoring of apoptotic process of five individual cells in three (low, medium, and high) etoposide concentrations at 9, 55, and 83 μ M, respectively.

FIG. 6. (a) Fluorescent image of cell nuclear DNA and (b) phase contrast image of morphology of HeLa-C3 cells. The line-shaped shadow in the phase contrast images is from the diffraction of light at two sides of the microchamber, which are curved under the influence of the valve control channels.

peaks of both fluorescent proteins. Another contributing reason is the capability of collecting light: Objective of a fluorescent microscope can certainly collect more light than a plate reader for a unit area.

We also compared the cell apoptosis assay results with the MTT assay; in living cells, the yellow colored MTT molecules are reduced to purple colored formazan products by the mitochondrial reductase enzymes. This color change is quantified by measuring the light absorbance at 595 nm by a spectrophotometer, which is commonly used as a standard colorimetric assay to determine the cytotoxicity of certain agents that may cause cell toxicity, metabolic dysfunction, and decreased performance. Figure 8(b) gives the results of MTT assay of etoposide on HeLa-C3 cells. Compared with the FRET assay shown above, the MTT assay gives similar trends. However, it shows slower response (decrease in cell viability) than the FRET assay. In the early stage of

FIG. 7. Averaged results of on-chip apoptosis assay. The relative Y/C ratio from YFP and CFP fluorescence images of each cell in all microchambers was recorded every 12 h for 2 days and averaged to give the relative Y/C ratio for each concentration at each time point. The number of individual cells recorded at each concentration is ~ 100 .

FIG. 8. Other assay methods of detection of apoptosis. (a) Caspase-3 activity assay with a 96-well plate. (b) MTT assay (the viability is relative to the control). All results are obtained from plate readers.

apoptosis, although caspase-3 is activated, the activity of the mitochondrial reductase enzymes, which the MTT measures, will mostly not be affected. Therefore, this cell viability test shows time delay in response to cytotoxic drug. The HeLa-C3 FRET system gives more accurate temporal sensitivity for real time quantifying the cell apoptotic process.

IV. CONCLUSIONS

We have developed a prototypic microfluidic platform for the generation of stepwise concentration gradients of drugs. We also demonstrated the application of this microsystem to study cell apoptosis by integrating into the chip a sensitive FRET-based cell apoptotic analysis method, which was previously demonstrated to directly quantify the apoptotic process by measuring the activity of caspase-3 under the influence of anticancer drug, etoposide. Sets of microfluidic valves are used to accurately meter different solutions and mix them to generate stepwise gradients of drugs to test their cytotoxicity with cell apoptotic assay. This microfluidic platform can provide several advantages: (1) Cytotoxicity of drugs at multiple concentrations can be simultaneously studied in parallel on one chip, thus saving time and labor and reducing systematic errors; (2) the sample size can be largely reduced; (3) microfluidic chambers facilitate long-time tracking of individual cells; and (4) microscopic imaging can give higher optical sensitivity than fluorescent scanner or UV-visible absorption. This microfluidic platform can also avoid possible shear force that exists on other cell-study microfluidic chips. Also, it resembles the traditional well plate format for drug screening, but with miniaturized sizes and higher throughput. This report is focused on the establishment of the microfluidic prototype for the study of cell apoptosis and we have tested five ladder channels on single chips; higher throughputs should be possible. This prototypic system should be useful in the areas of biology and bioengineering, especially for discovering apoptosis-inducing agents as potential anticancer drug candidates.

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