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Microfluidic Selection and Retention of a Single Cardiac Myocyte, On-Chip Dye Loading, Cell Contraction by Chemical Stimulation, and Quantitative Fluorescent Analysis of Intracellular Calcium

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A microfluidic method to study the contraction of a single cardiac myocyte (heart muscle cell) has been developed. This method integrates various single-cell operations as well as on-chip dye loading, and quantitative analysis of intracellular calcium concentration, $[Ca^{2+}]_i$. After the channel enlargement by on-chip etching to accommodate large-sized cardiac myoytes, a single cell is selected and retained at a V-shaped cell retention structure within the microchip. Owing to the fragile property of the cardiac myocytes that could easily be damaged by centrifugation, the calcium-sensitive fluorescent dye was loaded in the cell by on-chip dye loading. This on-chip method minimized the damage to the cells from the use of a centrifuge in the conventional method and provided a way of cellular analysis of fragile cells. Subsequently, quantitative analysis of $[Ca^{2+}]_i$ of a single cardiac myocyte by fluorescence measurement was achieved for the first time in a microfluidic chip, thanks to the intracellular calcium stimulant of ionomycin. The resting $[Ca^{2+}]_i$ of the cardiomyocyte determined was consistent with the literature value. From the spontaneous contraction study, it was found that fluorescence intensity cannot represent the $[Ca^{2+}]_i$ variation accurately, which implied the importance of the quantitative analysis of $[Ca^{2+}]_i$.

Muscle cell (myocyte) contraction allows high organisms to carry out and control crucial internal functions, such as the beating of the heart. If the cardiac myocytes (heart muscle cells) do not work well, it may lead to cardiovascular diseases.[1] Therefore, the contraction study of myocytes has attracted great interest.[2−4]

Calcium acts as a universal second messenger in a variety of cells. In the 1960s, Ebashi and Lipmann discovered in muscle fibers an intracellular Ca^{2+} storage site, the sarcoplasmic reticulum (SR).[5] Subsequently, they investigated the role of the Ca^{2+}-binding protein, troponin, in the contraction of striated muscle of higher vertebrates.[6] The Ca^{2+} release from the SR, or from the calcium channel in the cell membrane, will raise the cytosolic calcium concentration. When this concentration is high enough to saturate the Ca^{2+} sites on troponin C, contraction of the muscle cell will occur. If the intracellular calcium concentration decreases, the muscle cell will relax.[7] Therefore, intracellular calcium measurement is essential in the mechanistic study of cardiac activities,[8−10] and intracellular calcium measurement by fluorescence has become one of the most widely used approaches in the contraction study of myocytes.[11−15]

The dimensions (10−100 μm) of microfludic channels are highly compatible with the sizes of biological cells, which have made cellular assay a popular micro total analysis system application, as summarized in a recent review article.[16] Since the components in different cells or the cell at different stages are distributed differently, single-cell analysis has been conducted to identify the difference.[17−21]

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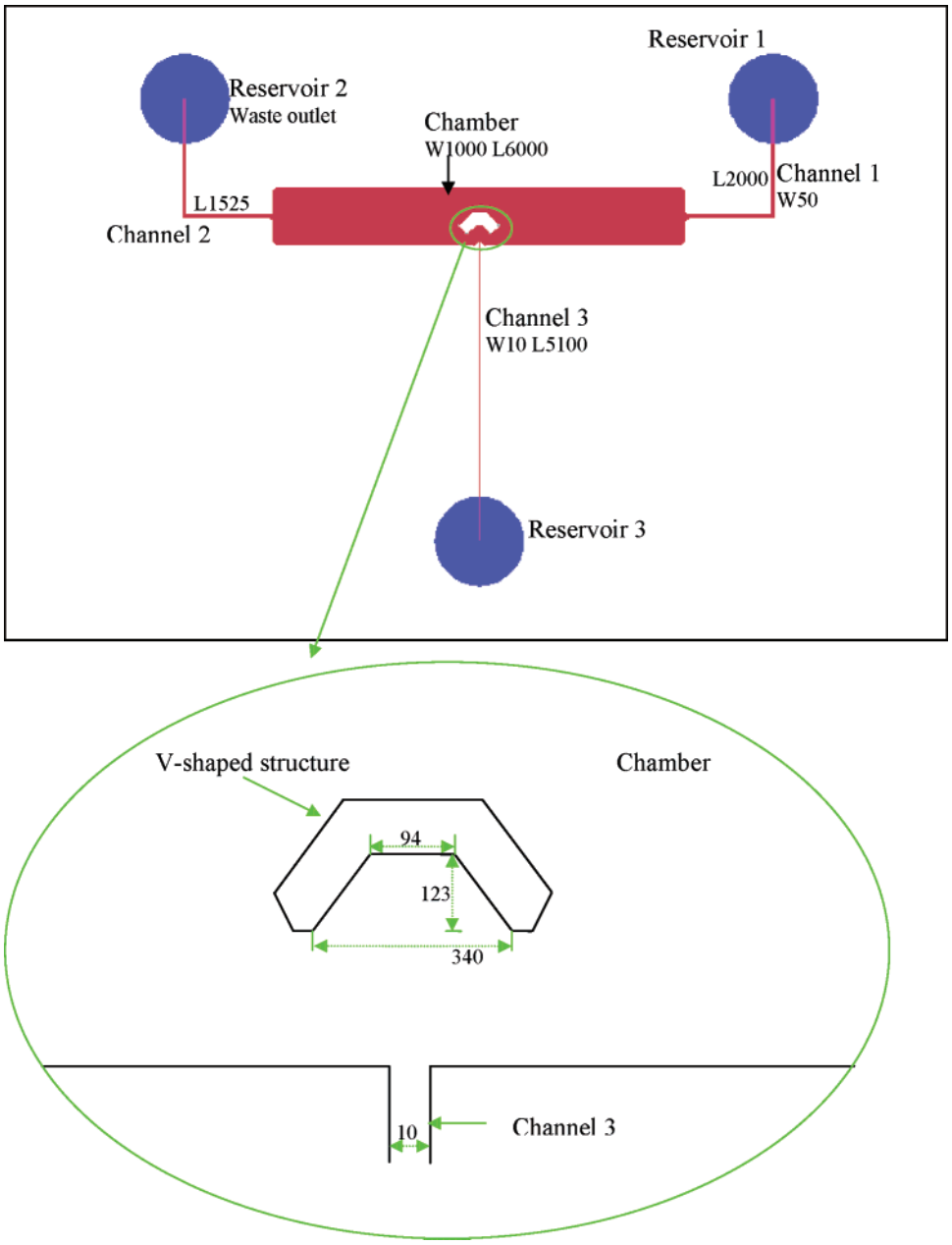

Figure 1. Design layout of the microfluidic chip. W, width. L, length (in μm). The widths of channels 1 and 2 are 50 μm, and that of channel 3 is 10 μm. The inset shows the dimension of the cell retention structure. All specifications are those on the photomask before wet etch.

Recently, measurements of intracellular calcium in mammalian cells were conducted in the microfluidic chip. Yang et al. designed a chip that has a parallel dam structure for docking of a group of HL-60 cells and monitoring of the ATP-dependent intracellular calcium uptake, but that structure cannot separate to get a single cell. [22] Later, Wheeler et al. developed a microfluidic chip to monitor the calcium flux of a single Jurkat T cell.[17] However, no quantitative analysis of $[Ca^{2+}]_i$ in on-chip cellular studies has ever been reported.

Several cellular studies on cardiac myocytes have been performed in the microfluidic chip. A microfluidic chip integrated with an acoustic wave sensor was fabricated for analyzing muscle cell contraction, but not at the single-cell level.[2] Werdich et al. reported one method to study single cardiac myocytes by recording the extracellular potential, but many cells indicated severe mechanical cell damage.[3] Recently, Kaji et al. reported an approach to study the gap junction between multiple micropatterned cardiac myocytes by measuring the cytosolic Ca^{2+} with fluorescence,[4] but the sensitivity was rather low (i.e., S/N ~3). Klauke et al. developed a planar microelectrode array to study the contraction of a single cardiomyocyte by electrical stimulation.[23] In all these reports, although fluorescent intensity was reported, no calibration was performed to quantify the concentrations of intracellular Ca^{2+}.

In this work, a microfluidic method to study the contraction of a single cardiac myocyte by intracellular calcium measurement has been developed. This method integrates single-cell selection, cell retention, dye loading, chemical stimulation, and quantitative

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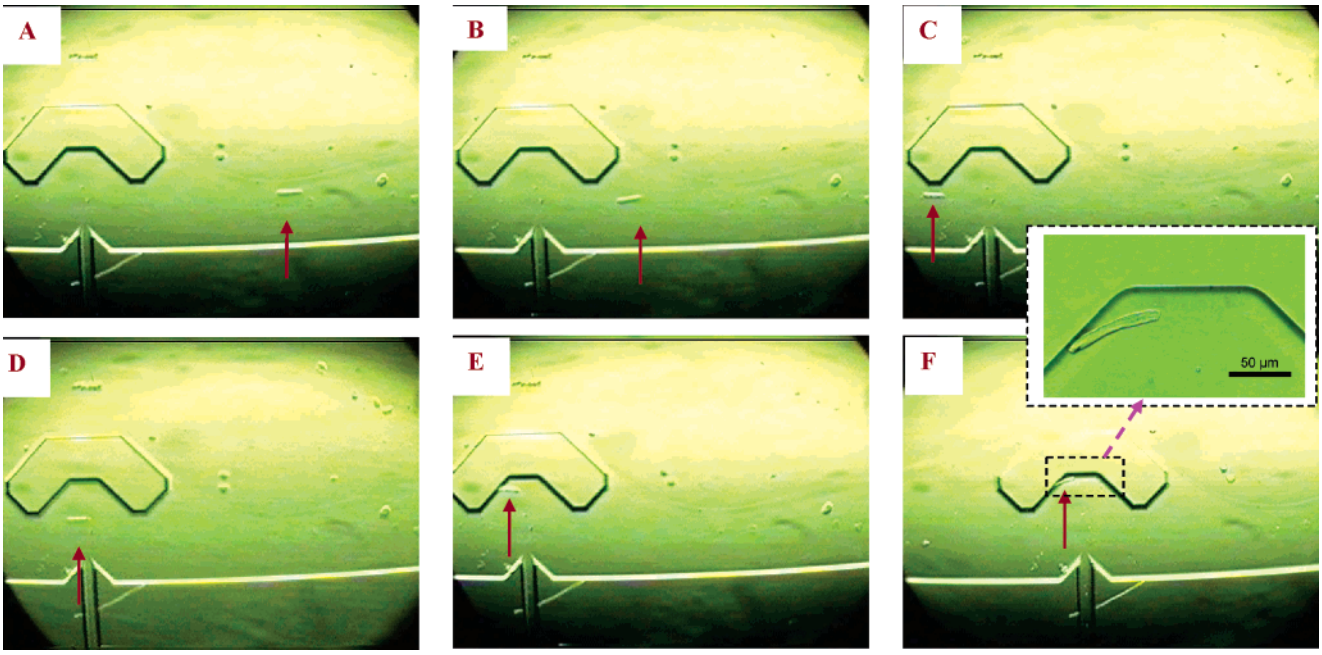

Figure 2. Selection and retention of a single cardiac myocyte shown by a series of bright-field images during the process as observed by a microscope (10×) using the phase contrast mode. (A, B) Cell suspension was put in reservoir 1 and the cell moved from the right to the left. (C, D) The liquid level of reservoir 1 or reservoir 2 was adjusted to transport the cell to stop at the entrance of the cell retention structure. (E, F) HBS solution was added to reservoir 3 to push the cell into the structure. Inset shows the cell retained in the structure in higher magnification.

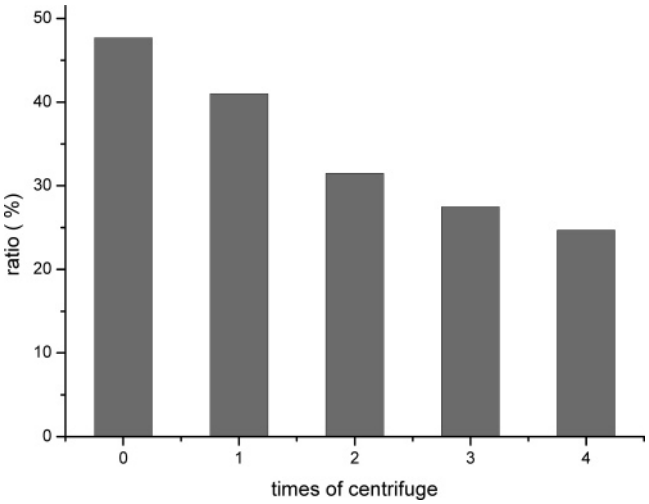

Figure 3. Cell damage effect of centrifuging to cardiac myocytes shown by the histogram of the change of the ratio of the number of good-shaped cells to that of the total number of cells. No Fluo-4 was loaded in these cells.

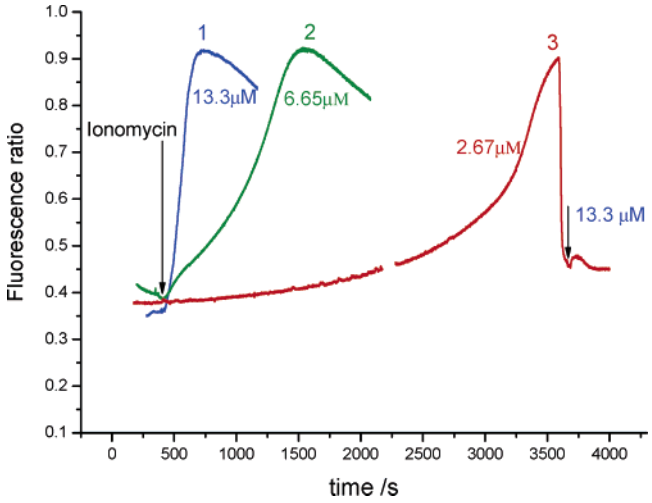

Figure 4. $[Ca^{2+}]_i$ flux in a cardiac myocyte stimulated by different concentrations of ionomycin. (1) 13.3, (2) 6.65, and (3) 2.67 μM ionomycin. For curve 3, at 3660 s, 13.3 μM ionomycin was introduced after 2.67 μM ionomycin. Normalization has been performed for easy comparison. The myocyte was on-chip loaded using 5.0 μM Fluo 4-AM solution.

analysis of intracellular calcium on one microfluidic chip. Due to the large size of the cardiac myocyte (cylindrical shape with a diameter of 15−25 μm and a length of 50−120 μm), we used an on-chip etching method to enlarge the channels of a bonded chip to accommodate the large-sized cardiac myocyte. Due to the fragile properties of cardiac myocyte and the fact that the rod-shaped and large-sized cardiac myocyte is easily damaged by the centrifuge, an on-chip dye loading approach was used to minimize the damage to the cells from the centrifuge in the conventional method. So the on-chip dye loading provided a means for cellular analysis of fragile cells. What is more, as far as we know, no calibration of intracellular calcium of a single cell by a microfluidic method was ever reported. For the first time, we developed a microfluidic method to determine the concentration of intracellular calcium of a single cardiac myocyte quantitatively.

EXPERIMENTAL SECTION

Chip Fabrication and Characterization. The chip was fabricated through the Protolyne Chip program of Canadian Microelectronic Corp. (CMC). The general microfabrication procedures include standard RCA cleaning, thin-film deposition, photolithography, wet HF etching, access hole forming, and chip bonding.[24]

The schematic diagram of the layout of the microfluidic chip is shown in Figure 1.[18] In this chip, there are three reservoirs,

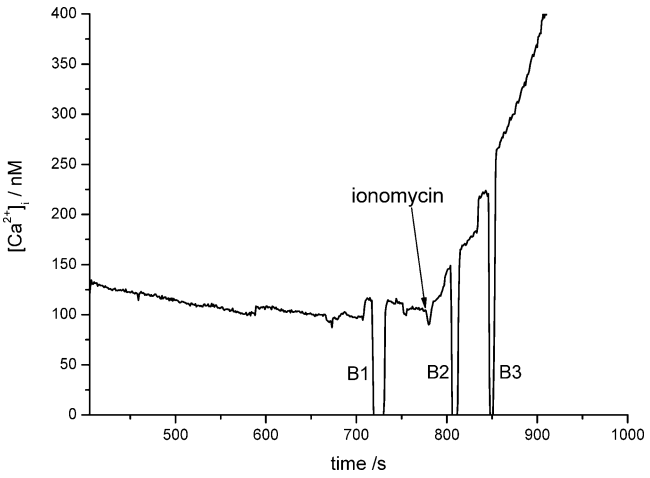

Figure 5. Quantitative analysis of intracellular calcium of a single cardiac myocyte in the cell retention structure ($[Ca^{2+}]_i$ versus time curve after the conversion from the fluorescence intensity data using eq 1). At 776 s, 13.3 μM ionomycin was introduced via channel 3. B1−B3, background.

three channels, one chamber, and one cell retention structure. Reservoir 1 and reservoir 2 are for cell inlet and waste outlet, respectively. Reservoir 3 is used to deliver reagents. The cell retention structure is in the form of a V-shaped barrier with a central stretch. The width of channels 1 and 2 is 89.2 μm at the top because of undercut of etching and 50 μm at the bottom of the channels; whereas the width of channel 3 is 49.2 and 10 μm at the top and the bottom, respectively. Other dimensions are shown in Figure 1.

Reagents. Fluorescent calcium probe Fluo-4 AM ester (50 μg, special packaging, Molecular Probes, Eugene, OR) was first dissolved in 50 μL of dimethyl sulfoxide (>99.9%, Sigma-Aldrich Co., St. Louis, MO) to make a stock solution. Note that Fluo-4 AM is light sensitive and must be stored in the dark at −20 °C. Before use, it was diluted in Hanks' balanced salt solution (HBS, Invitrogen Corp., Grand Island, NY) to make a 5.0 μM working solution. Ionomycin (calcium salt, Sigma Chemical Co.) was used for chemical stimulation of cells and to saturate the Ca^{2+}−Fluo-4 fluorescence within cells. All other reagents were of analytical grade.

Instrumentation. An optical observation and imaging system and fluorescent measurement system was used as previously described.[18] Briefly, an inverted microscope (TE300, Nikon, Mississauga, ON, Canada) was connected to a video camera (JVC, TK-C3180). A TV set and VCR system (JVC) was used for easy microscopic observation and video recording. Red light using a 600-nm long-pass filter was used for optical cell observation, without interfering with the fluorescent measurement. A video capture card (ATI-TV wonder bt878, Markham, ON, Canada) was installed in a personal computer for image capture either from video recordings or directly from the camera.

Fluorescence excitation was achieved by a xenon arc lamp (PTI, Photon Technologies International, London, ON, Canada). The excitation wavelength of 480 nm was selected by the monochromator (PTI). The fluorescent emission at 525 nm (Omega Filters) was measured by a microphotometer, which consisted of an adjustable aperture and a photomultiplier tube (PMT, PTI) coupled to the side port of the microscope. Photometeric data were collected and processed by a software of Felix (PTI). Using a dichroic filter (580 nm), only red light for cell observation went to the video camera; whereas green fluorescent emission went to the PMT.

In the fluorescence system, we fixed a rectangular aperture that was slightly larger than the cell size. In the imaging system, a similarly sized rectangle was marked on the TV screen. Therefore, the chip could be translated to let the cell move in or out of the aperture.

Procedure of On-Chip Etching of a Bonded Chip. To enlarge the channels of a bonded chip, a small amount of 12% HF (Caledon Laboratories Ltd., Georgetown, ON., Canada) solution was put into reservoirs 1 and 2 (see Figure 1 for notations) for etching. Since channel 3 was not used for cells, it was not etched and so only water was put into reservoir 3. After etching for 45 min, all the solutions within the microchannels were removed by liquid suction to a special container for safe disposal. The channel width was examined under the microscope in order to estimate the channel depth. If the channel was not etched deep enough, the etching process of HF was repeated. When handling HF, special safety precautions must be taken.

Isolation of Cardiac Myocytes. Ventricular myocytes were isolated from the heart tissues of New Zealand white rabbits (of either sex) by Dr. Glen Tibbits' group using a previously described method.[25] Briefly, a rabbit was first intraperitoneally injected with pentobarbital and heparin, and then the heart was rapidly excised in a 4 °C Ca^{2+}-free solution. The heart was then perfused in the Langendorff mode: first with collagenase (Yakult) and then with protease (Sigma). The ventricles of the heart were subsequently removed, chopped into small pieces, and washed twice using the storage solution [120 mM L-glutamic acid monopotassium salt ($C_5H_8NO_4K$), 5 mM $MgCl_2$, 20 mM taurine, 1 mM EGTA, 10 mM glucose, 10 mM HEPES].

Off-Chip Dye Loading Procedure. For the off-chip dye loading, a centrifuge was used four times. A cell suspension (1.5 mL) was first transferred to a 1.5-mL centrifuge vial, and the muscle cells were allowed to settle for 3−5 min before the cell suspension was centrifuged at 1000 rpm for 1.5 min. After removing the supernatant, 350 μL of 5.0 μM Fluo-4 AM ester solution was added to the cell pellet in the vial, and the cell suspension was agitated gently. The vial was kept in the dark for 30−40 min at room temperature to complete dye loading into the cells. To remove the excessive external dye after dye loading, the vial was centrifuged again at 1000 rpm for 1.5 min. After removing the supernatant, the cell pellet was resuspended in 1000 μL of HBS solution before another centrifuging and resuspending was repeated once. Then the forth centrifuging was applied for removing the supernatant before a proper amount (e.g., 0.4 mL) of HBS was added to the cell pellet for experiment.

RESULTS AND DISCUSSION

Channel Enlargement of a Bonded Chip by Multiple On-Chip Etching. A single rabbit cardiac myocyte has a cylindrical

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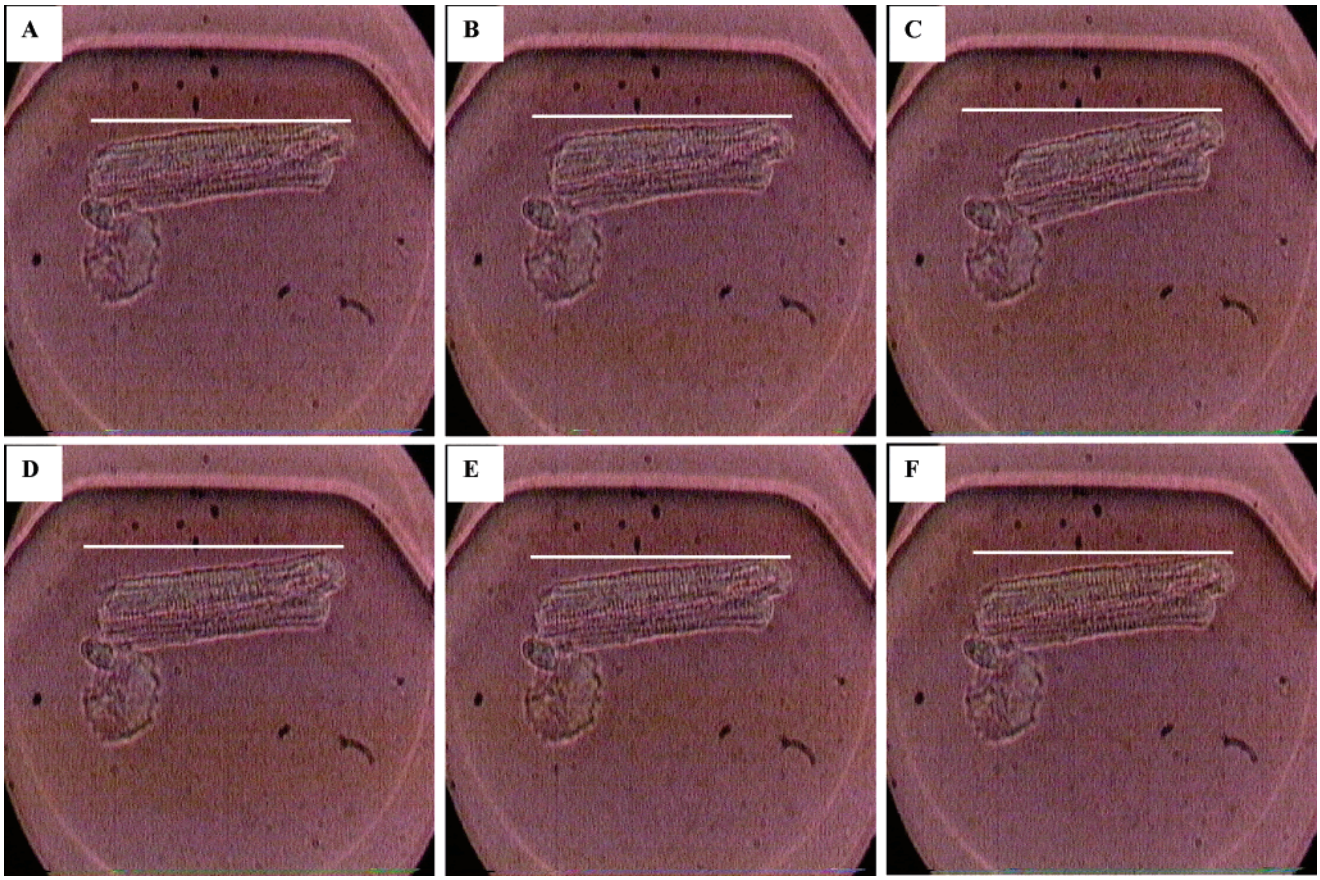

Figure 6. Cycle of contraction and relaxation of the cardiac myocyte in the microfluidic chip. (A−C) Cell contraction process. (C−F) Cell relaxation process. The round body beside the rectangular muscle cell is cell debris that was there before the introduction of cell suspension. The length of the white bar is 103 μm, which is equal to that of the muscle cell in (A).

shape with a diameter of 15−25 μm and a length of 50−120 μm, but the chip that we obtained is limited to a channel depth of ~20 μm and the width at the channel bottom is only 50 μm, which allows only small cells (e.g., 3-day-old rabbit cells) to go into the channels. Most of the larger adult cells cannot go into the channel, and they will jam at the inlet of the channel or the channel corner.

Therefore, we used multiple on-chip HF etching method (4 times) to enlarge the channels of the bonded chip to accommodate the large-sized cardiac myocytes. After HF etching, the new channel depth was determined to be ~35 μm and the width at the top and the bottom of channel cross section to be ~120 and 80 μm, respectively. This new depth proved to be sufficient in our experiments because the cells were found to easily go into the channel and the chambers, and no jamming of the channel happened.

During on-chip etching, there was no stirring in the channel, which was undesirable for the removal of etch reaction products. Therefore, etching was repeated a few times in order to achieve the desired depth. As for the etch quality, the channel wall surface was found to be quite smooth and no pits were noticed under microscope.

Optimization of Excitation Wavelength of Fluo 4. An excitation scan was carried out to optimize the excitation wavelength of Fluo 4 loaded into a single cardiac myocyte. After subtraction of the fluorescence background from the glass chip, we get the fluorescence solely from Fluo 4. The maximum excitation occurred at 478 nm, which was slightly different from the literature value of 494 nm (emission 516 nm) in which Fluo 4 was measured in solution, but not in cells.[26] This discrepancy may be caused in part by the absorption/scattering by intracellular constituents,[27] changes in the polarity,[28] or the viscosity [29] of the environment. Actually, we chose 480 nm as the optimal excitation wavelength in our experiments in order to be apart from the glass background.

Single-Cell Selection and Retention. Here, we used the cell retention structure, which is a V-shaped barrier with a central stretch in the chamber, to select and retain a single cell. A series of images captured from the video recording to depict these events of selection and retention of a cardiomyocyte were shown in Figure 2. In Figure 2A and B, the cell was moving from the right to the left after the cell suspension was added from the right. In Figure 2C, the cell moved further and passed the entrance of the cell retention structure. In Figure 2D, the cell moved back and stopped at the entrance by adjusting the liquid levels of reservoirs 1 and 2. The cell was pushed into the cell retention structure (Figure 2E and F) when a flow was induced via the central reagent channel. The inset shows the cell retained in the structure as observed at a higher magnification (40×).

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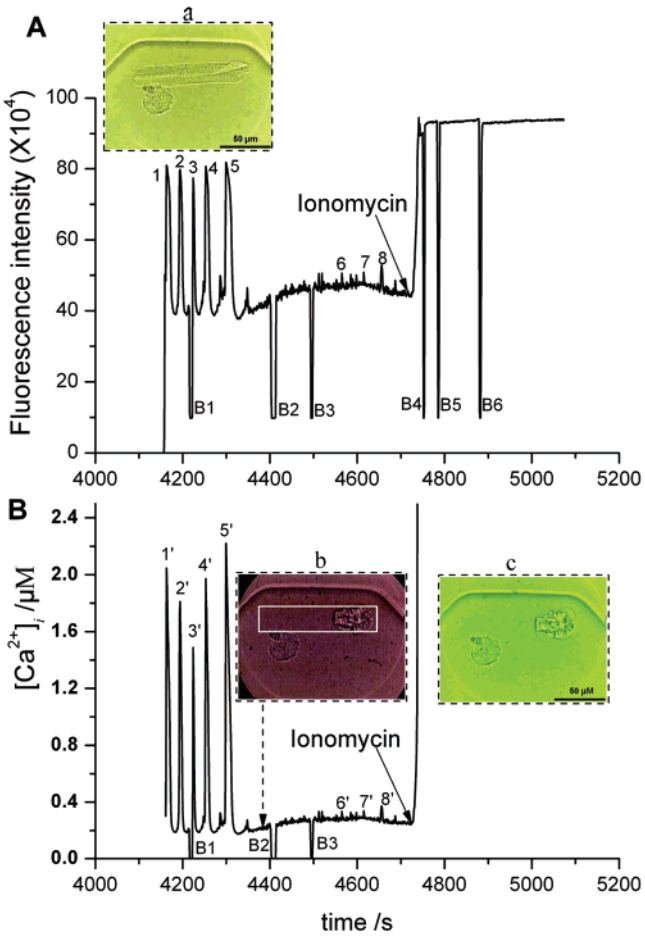

Figure 7. Periodical contraction and relaxation of a cardiac myocyte in the microchip. (A) Fluorescent intensity versus time curve. (B) $[Ca^{2+}]_i$ versus time curve. At 4722 s, 13.3 μM ionomycin was introduced. B1−B6 show the times when background measurements were made. Inset a shows the white light bright-field image of the cardiac myocyte in the cell retention structure before the experiment. Inset b shows the red light bright-field image of the cardiac myocyte at 4384 s. An observation window used for fluorescent measurement is shown as a white rectangle. Inset c shows the white light bright-field image of the cardiac myocyte after the experiment.

On-Chip and Off-Chip Dye Loading. Conventionally, off-chip dye loading of a Ca^{2+} probe is widely used for intracellular calcium measurement. In this method, after the first centrifugation for removal of the supernatant (storage solution), a Fluo-4 AM solution was added for dye loading into the cells. If we want to monitor the off-chip loading process, the cell suspension with Fluo-4 AM solution could be put on a glass slide. But due to liquid evaporation, the measurement cannot take too long because the liquid will dry up, the cell will die, and the fluorescence signal will be affected. Hence the chamber in our microfluidic chip was used to monitor the off-chip dye loading process, and the evaporation problem was resolved.

Moreover, during off-chip dye loading, at least four centrifugations have to be used to remove the storage solution and dyes. This is not only tedious but also harmful to the fragile heart muscle cells, whose long cylindrical shape and fragility make the cells easily damaged by the centrifuge operation. This damaging effect to the cells has been studied as well. We found before centrifuging, many cells were in good long cylindrical shape, with only a few dead cells in the shape of round bodies. As the number of centrifugations increased, the number of healthy cells decreased and the number of round bodies increased. These results suggested that the cells had suffered increased damage due to centrifuging. This happened even though we used a low centrifuge speed (1000 rpm) and a short duration (1.5 min). If we compared the appearance of the cells before centrifuging to that of the cells after four centrifugations, we observed that many cells had been damaged and became round bodies. The histogram in Figure 3 shows the change of the ratio of the number of the good cells to that of the total cells after centrifuging. The ratio deteriorated from 48 (before centrifuging) to 24% after four centrifugations. This means that half of the muscle cells have suffered serious cell damage.

Owing to the disadvantages of off-chip loading, we developed an on-chip dye loading method to minimize the damage to the cells. Immediately after washing the heart muscle cell retained in the cell retention structure, Fluo-4 AM solution was introduced for on-chip dye loading. Meanwhile, fluorescence measurement was used to monitor the on-chip dye loading process. The fluorescence was caused by the hydrolysis of Fluo-4 AM into Fluo 4 and the binding of Fluo 4 to the basal level of Ca^{2+} in a relaxed muscle cell. From the fluorescence results, we found that 40−50 min (or 2400−3000 s) were enough for on-chip dye loading. After washing, another 10 min were used to complete the hydrolysis of the Fluo-4 AM ester inside the cells.

Real-Time Monitoring of Ionomycin-Stimulated $[Ca^{2+}]_i$ Flux. Mobilization of intracellular Ca^{2+} (a secondary messenger in cell signaling) is related to many cardiac activities such as the contraction and relaxation of the cardiac myocyte. A variety of stimuli, such as ionomycin,[17] ATP,[22] and glucose,[20] have been used to elicit Ca^{2+} responses in microchip cell experiments. In this work, ionomycin was used to stimulate the $[Ca^{2+}]_i$ flux of a single cardiac muscle cell.

After the on-chip dye loading, ionomycin (13.3, 6.65, and 2.67 μM) was introduced via the reagent channel for stimulation, and the results are shown in Figure 4. In all curves, the increase of fluorescence intensity was observed, which represents the increase of $[Ca^{2+}]_i$. The stimulation has also caused the myocytes to contract into round bodies, and the fluorescence intensities have achieved their own maximum. At a higher concentration of ionomycin (13.3 μM, curve 1), a faster $[Ca^{2+}]_i$ response was observed, which took only ~310 s to achieve its maximum. At lower concentrations of ionomycin (such as 6.65 and 2.67 μM), the cardiac myocyte gave slower response and it took a longer time for the $[Ca^{2+}]_i$ flux to achieve the highest level than 13.3 μM ionomycin did. For instance, the use of 2.67 μM ionomycin (curve 3) took ~3190 s (10 times that of 13.3 μM) to achieve the maximum while the use of 6.65 μM ionomycin only took ~1530 s (curve 2). Therefore, it can be seen that the ionomycin-stimulated $[Ca^{2+}]_i$ response is a concentration-dependent process.

It is well known that this observation is a store-regulated calcium uptake process and the calcium increase is derived from the internal store, SR, if the buffer is a Ca-free solution.[30−32] After ionomycin permeated into the myocyte, it acted at the SR to

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release Ca^{2+}, which would quickly combine with free Fluo-4 in the cytosol.[33] After the maximum, a decrease in $[Ca^{2+}]_i$ was observed, which may be due to the efflux of Ca^{2+}, the dye, or both, as we previously reported on yeast cells.[20]

Since 13.3 μM ionomycin can easily saturate the fluorescence within a short time, it has been used in the subsequent experiments in which a stimulant is applied to provide the maximum fluorescence for quantitative analysis of intracellular calcium of a single cardiomyocyte.

Quantitative Analysis of Intracellular Calcium of a Single Cardiac Myocyte. In this work, the intracellular calcium concentration of a single cardiac myocyte in the microfluidic chip was quantified for the first time.

The total fluorescence (F) when the cell was in the aperture, and the background fluorescence (F_{min}) when the cell was out of the aperture, were measured. At the end, to saturate Fluo-4 for calibration, 13.3 μM ionomycin was introduced to reach the cellular fluorescence maximum (F_{max}). Accordingly, the intracellular calcium concentration can be determined by the following equation,[12]

$$[Ca^{2+}]_i = K_d \frac{F - F_{min}}{F_{max} - F} \quad (1)$$

where F is the fluorescence intensity, F_{min} is the background fluorescence determined from a cell-free area in a calcium-free solution, F_{max} is the maximum fluorescence acquired, K_d is the dissociation constant of Fluo-4, and a value of 0.35 μM was used for calculations.[26]

According to eq 1, $[Ca^{2+}]_i$ at different times were calculated from the fluorescence intensity data and the result of the intracellular calcium measurement ($[Ca^{2+}]_i$-t curve) was shown in Figure 5. It can be found that, before addition of ionomycin, the baseline fluctuated slightly, which is indicative of intracellular calcium mobilization. After adding 13.3 μM ionomycin, the cellular fluorescence due to Fluo-4 was saturated and achieved the maximum, which was similar to curve 1 shown in Figure 4. To obtain the background fluorescence (F_{min}), the chip has been translated so that the cell is out of the aperture window, resulting in some dips (B1−B3). It can been seen that the $[Ca^{2+}]_i$ level of this cardiac myocyte at resting state was ~100 nM, which agrees very well with the literature value of ~100 nM.[7]

The spontaneous contraction of a cardiac myocyte in the cell retention structure was monitored too. The cell was observed to contract and relax periodically. Figure 6 shows one cycle of the contraction and the relaxation of the cardiac myocyte in the cell retention structure. Panels A−C in Figure 6 show the contraction process of the cardiac myocyte, and the cell in Figure 6C is the shortest in length. Panels C−F in Figure 6 show the relaxation process. (See the movie file contract.avi in the Supporting Information about this contraction and relaxation process.) The inset a (before experiment) and inset c in Figure 7 (after experiment) show the cell shape change during this experiment.

During cell contraction, the fluorescence signal was also measured as shown in Figure 7A. From 4157 to 4325 s, there are five fluorescence peaks that were caused by five cycles of contraction and relaxation of the cardiac myocyte. After 4325 s, the cardiac myocyte stopped periodic contractions and remained in the contracted status (see inset b). However, we still observed some calcium spikes (such as peaks 6−8) during this process, which indicated that there were calcium fluxes regulated by SR, though no obvious cell shape change was observed. The origin of these small spikes is under further investigation. At ~4724 s, 13.3 μM ionomycin was introduced to saturate the cellular fluorescence due to Fluo-4. B1−B6 was to measure the background fluorescence determined from a cell-free area in a calcium-free solution.

After calibration using eq 1, the fluorescence intensity is converted to $[Ca^{2+}]_i$, as shown in Figure 7B. Peaks 1′, 2′, 3′, 4′,- and 5′ are the calcium peaks corresponding to the fluorescence peaks in Figure 7A due to the spontaneous muscle cell contraction. It can be seen that $[Ca^{2+}]_i$ of this adult cardiac myocyte at resting status is ~180 nM. Once contracted, the intracellular calcium concentration increased to ~2.0 μM. This change is consistent with the contraction mechanism of cardiac myocyte regulated by calcium that binds to the contractile protein, troponin C.[7]

From Figure 7B, it can also be found that, during contractions, the $[Ca^{2+}]_i$ peak intensity varies appreciably, ranging from 1.8 (peak 2′) to 2.2 μM (peak 5′) (peak 3′ was not included since the contraction happened nearly when the background measurement was carried out). If we look into the $[Ca^{2+}]_i$−t curve (Figure 7B) and F−t curve (Figure 7A), it can be found that there are more variations among the five peaks in the $[Ca^{2+}]_i$−t curve than those in F−t curve. The variation in $[Ca^{2+}]_i$ was as high as 36%, relative to the highest peak 5′. Note that this variation is not as obvious in Figure 7A, which only depicts a maximum variation of 12% in fluorescent intensity. Such a higher variation in $[Ca^{2+}]_i$ is the direct consequence of performing quantitative $[Ca^{2+}]_i$ measurement after calibration using eq 1. From the comparison, it can be seen that the F−t curve cannot accurately represent the exact $[Ca^{2+}]_i$ variation, which implies the importance of quantitative analysis of $[Ca^{2+}]_i$. This method is commonly used in conventional calcium measurements, though to the best of our knowledge, this method is used for the first time in microfluidic cell experiments. Quantitation of $[Ca^{2+}]_i$ using ratiometric dyes, such as Indo 1, which provides higher accuracy in $[Ca^{2+}]_i$, will be pursued in future work.

CONCLUSION

In this work, we developed a microfluidic method to study the contraction of a single cardiac myocyte. This method integrates on the chip the following operations: single-cell selection, cell retention, dye loading, chemical stimulation, fluorescence measurement, and quantitative analysis of intracellular calcium concentrations.

Our on-chip dye loading method can minimize the damage to the cells by avoiding the use of the centrifuge. This provides an improved method to perform cellular analysis of fragile cells. For the first time, we have quantitatively determined the intracellular calcium concentration of a single cardiac myocyte with a microfluidic approach, thanks to the use of the calcium-free background fluorescence as F_{min} and ionomycin-saturated cellular fluorescence as F_{max} for calibration using eq 1. This work will have wide

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applications in the field of intracellular calcium measurement and contraction studies of cardiac myocytes, such as single-cell screening of heart disease drugs.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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