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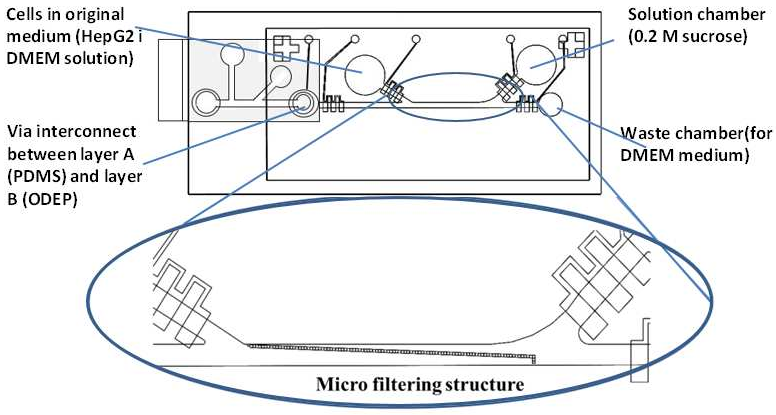

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Optically-induced dielectropheresis sorting with automated medium exchange in an integrated optofluidic device resulting in higher cell viability*

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We demonstrated the integration of a microfluidic device with an optically-induced dielectrophoresis (ODEP) device such that the critical medium replacement process was performed automatically and the cells could be subsequently manipulated by using digitally-projected optical images. ODEP has been demonstrated to generate sufficient forces for manipulating particles/cells by projecting a light pattern onto photoconductive materials which creates virtual electrodes. The production of the ODEP force usually requires a medium that has a suitable electrical conductivity and an appropriate dielectric constant. Therefore, a 0.2 M sucrose solution is commonly used. However, this requires a complicated medium replacement process before one is able to manipulate cells. Furthermore, the 0.2 M sucrose solution is not suitable for the long-term viability of cells. In comparison to conventional manual processes, our automated medium replacement process only took 25 minutes. Experimental data showed that there was up to a 96.2% recovery rate for the manipulated cells. More importantly, the survival rate of the cells was greatly enhanced due to this faster automated process. This newly developed microfluidic chip provided a promising platform for the rapid replacement of the cell medium and this was also the first time that an ODEP device was integrated with other active flow control components in a microfluidic device. By improving cell viability after cell manipulation, this design may contribute to the practical integration of ODEP modules into other lab-on-a-chip devices and biomedical applications in the future.

1. Introduction

In the past decade, multiple microfluidic devices have been integrated into a single chip such that a complete protocol for analysis and diagnosis can be automatically implemented by utilizing integrated microfluidic systems.[1-2] The manipulation and separation of micro- and nano- particles are essential for a variety of biomedical applications, for example, sample pretreatment, cell manipulation/separation and detection protocols.[3-6] Cells and micro-particles in bio-samples can be separated according to their size differences, electrical properties and other physical properties.[7-10] Recently, methods to use the optically-induced dielectrophoresis (ODEP) force have attracted considerable interest and made a substantial impact, particularly as a promising technique to manipulate and separate cells and micro-particles in biomedical applications. The traditional complicated process of fabricating micro-electrodes to generate the dielectrophoresis (DEP) force can be substituted by using "virtual" electrodes defined by a projected light pattern.[11-12] With the ability to manipulate a single cell or particle by using appropriate optical patterns, ODEP has been developed as an enabling technique particularly for the manipulation, separation, collection, alignment, transportation and characterization of cells/particles[13].

In order to manipulate cells, a proper medium (such as a 0.2 M sucrose solution) was commonly used to generate sufficient ODEP forces[15]. However, the cells had to be centrifuged in advance in order to replace the original medium, This required a complicate medium replacement process before one could manipulate cells. Furthermore, the 0.2 M sucrose solution is not suitable for long-term survival of the cells. Thus another medium replacement process has to be performed after ODEP manipulation. Therefore, there is a great need to develop a new technique to address this issue in order to make ODEP-based manipulation practical for use in clinical laboratories.

In this work, we demonstrated the integration of microfluidic devices with an ODEP device such that the critical medium replacement process was automated and the cells could be subsequently manipulated using projected optical images. This integrated microfluidic chip may provide a platform for fast and automated exchanges of cell mediums. It is also the first time that an ODEP device has been integrated with microfluidic-based control devices.

2. Materials and Methods

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2.1 Device Design and Fabrication

This integrated microfluidic chip must perform the following four critical steps for automatic medium replacement, including (a) injection of cells without pre-treatment, (b) replacement of the medium in the chip, (c) applying the ODEP force to manipulate the cells, and (d) harvesting the cells from the chip after another medium replacement process. Note that the manipulated cells must remain viable after harvesting. In order to enhance the survival rate of cells after ODEP manipulation, it is essential to reduce the total time necessary to perform the complete experimental protocol. For instance, for the HepG2 cell line, when immersed in the 0.2 M sucrose solution for over 50 minutes could result in a survival rate of only 3.4% for these cells (from our own experimental data). Therefore, an automated microfluidic device was propsed to implement the aforementioned processes.

The integrated microfluidic chip was designed with two modules including a polydimethylsiloxane (PDMS) microfluidic control module which performs the medium replacement function, and another ODEP module located underneath the PDMS microfluidic control module, which was used to manipulate cells (Figure 1). An exploded view of the integrated microfluidic chip (Figure 1a) shows that it is composed of an ODEP layer (including a double-sided tape and an amorphous silicon (a-Si) layer deposited on top of a bottom indium-tin-oxide (ITO) glass), a top ITO glass and an PDMS layer. Note that the PDMS layer contains an air chamber layer and a liquid channel layer. The air chambers are used to pump liquid in the liquid channels located underneath. The liquid channels are used to transport the cells and the mediums. Two ITO glass slides are used as the substrates for this integrated microfluidic chip and could be used for ODEP operation. A uniform electric field is first established between the top ITO glass and the bottom ITO glass (coated with a thin layer of a-Si) separated by a patterned double-sided tape as a spacer (30 μm thick) to define microchannels. When the light illuminates the photoconductive layer (a-Si) deposited on top of the bottom ITO glass, the conductivity of the illuminated area may increase four to five orders of magnitude. This induces a local, non-uniform electric field such that particles/cells may experience attractive or repulsive ODEP forces, depending on the conductivity and permeability of the particles/cells relative to the surrounding medium. Therefore, live and dead cells which experience different ODEP forces may be separated using this approach[14].

A micro filtering structure, which is designed and patterned in the PDMS microfluidic control module to perform medium replacement, is depicted in Figure 1(b). The micro filtering structure, consisting of a serial of angled columns (125 μm by 125 μm squares with each 40 μm tall) with 10 μm spacings, was designed with an inclination angle of 10° with respect to the microchannel. A scanning electron microscope (SEM) photograph of the micro filtering structure is shown in Figure 1(c). It could be used as filtering structures to separate cells and medium for the medium replacement process. In addition, the microfluidic control module integrates micropumps and microvalves to automate the medium replacement process. With this approach, cell viability can be greatly improved since the entire process could be automated within a shorter period of time. Figure 1(d) shows a photograph of an assembled integrated micro-optofluidic device. The dimensions of the assembled device is approximately 8.0 cm long x 3.5 cm wide. The ITO glass middle layer is precisely 8.0 cm long x 3.5 cm wide. The transparent PDMS layers above the ITO glass layer are slightly narrower (approximately 3.0 cm wide) and about 5.0 cm long. The ITO glass chip with the opaque amorphous silicon thin-film layer is approximately 2.0 cm wide and 5 cm long. However, this bottom part of the ODEP device is offset from the glass middle layer in order to expose some amorphous silicon for electrical contact. Hence the overall length of the assembled device is a little longer than 8.0 cm.

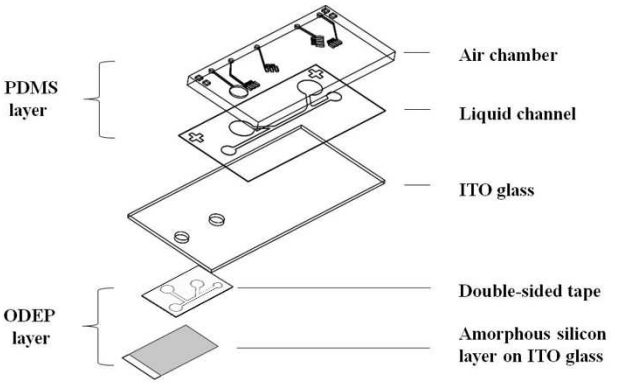
The microfabrication process is described as follows. Briefly, the liquid channel layer and the air chamber layer of the PDMS microfluidic control module were fabricated using PDMS (Sylgard 184A and Sylgard 184B, Sil-More Industrial Ltd., Taiwan) and a standard soft lithography replica molding method[16]. The mold for the liquid channel layer was fabricated using SU-8 (Microchem, USA) photoresist on a silicon wafer. The mold for the air chamber layer was made using a computer-numerical-control (CNC) machining process to pattern a polymethyl methacrylate (PMMA) plate. PDMS was prepared by mixing the silicone elastomer and the elastomer curing agent in a 10:1 ratio by weight. The PDMS was then poured onto the PMMA mold of the air chambers. PDMS was also poured onto the SU-8 mold which defined the liquid channels and spin-coated at 2000 rpm for 30 seconds to obtain a thickness of 60 μm. The PDMS layer defining the air chambers were manually peeled-off from its mold afterwards. Because the liquid channel layer was extremely thin, this layer was first bonded to the air chamber layer before it could be de-molded from the SU-8 mold. An oxygen plasma was used to irreversibly bond the PDMS liquid channel layer to the PDMS air chamber layer, and later, the entire PDMS structure to the ITO glass substrate. Finally, another ITO glass with the a-Si layer was bonded to the bottom side of the ITO glass layer by patterned double-sided tape which also defined the ODEP microchannels.

2.2 Device Operating Procedure

The operating process of the integrated microfluidic device is schematically illustrated in Figure 2(a). First, cells (HepG2, which is from a human liver carcinoma cell line) suspended in the original culture medium (Dulbecco's Modified Eagle Medium, DMEM, Life Technologies, USA) were injected into a sample chamber of the PDMS microfluidic control module (Figure 2a(I)). The cells and the culture medium were then pumped by the micropump towards the micro filtering structure such that the cells were retained by the filter and the culture medium flowed through the filter; thus achieving cell separation (Figure 2a(II)). Then a 0.2 M sucrose solution, necessary for the efficient generation of the ODEP force, was injected into the same chamber and perfused the cells for a specific period of time which will be detailed in the next section. Next, additional 0.2 M sucrose solution was pumped from the opposite direction to transport the cells into the ODEP module (Figure 2a(III)). The cells were then manipulated or separated by virtual electrodes that were produced from optically-projected images which selectively illuminated the photoconductive layer (a-Si) of the ODEP chip

(Figure 2a(IV). In this study, a series of moving light patterns, which activated the ODEP force specific for live cells, were adoptd to push the live cells such that dead/live cells could be separated[14]. After manipulation by the ODEP force, cells were extracted along with the 0.2 M sucrose medium diluted and mixed with the original cell culture medium (DMEM). This cell mixture was then subsequently cultured in bench-top incubators for downstream analysis. The micropumps and the microvalves were pneumatically driven by applying compressed air. The pumping rate could be precisely regulated by the driving frequency of the electromagnetic valves and applied air pressures. Detailed information about the micropump and the microvalves can be found in our previoius work[15].

There are two key design principles for the micro filtering structure in the microfluidic control module. The first involves the size difference between the cells and the gap spacing in the filtering structure, and the second involves the direction of the flow. In this micro filtering structure, the space between the two square structures is 10 µm, whereas the size of an average living HepG2 cell is ~15 µm. Due to their larger size, the cells were trapped by the filter and the medium continued to flow through the filter and into the waste chamber. Then, more 0.2 M sucrose solution was pumped in the opposite direction from the solution chamber into the microchannel. This 0.2 M sucrose solution would flush cells trapped by the filtering structure and transport these cells to the ODEP layer for subsequent manipulation (Figure 2b).



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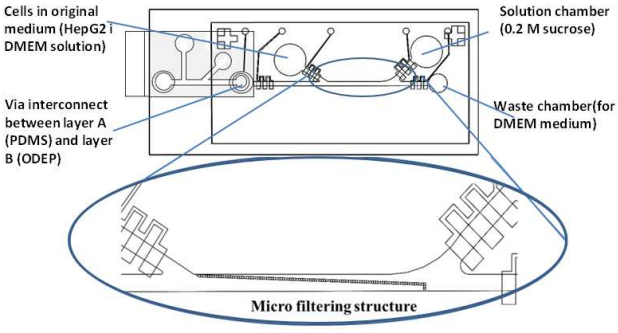

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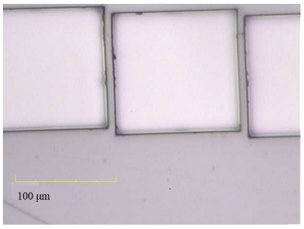

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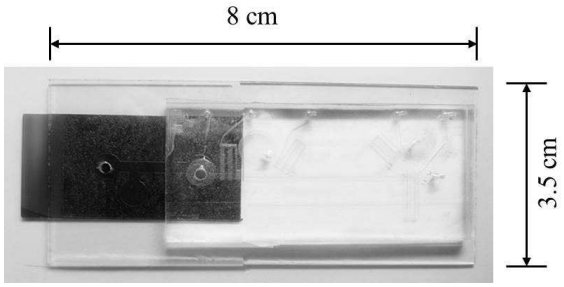
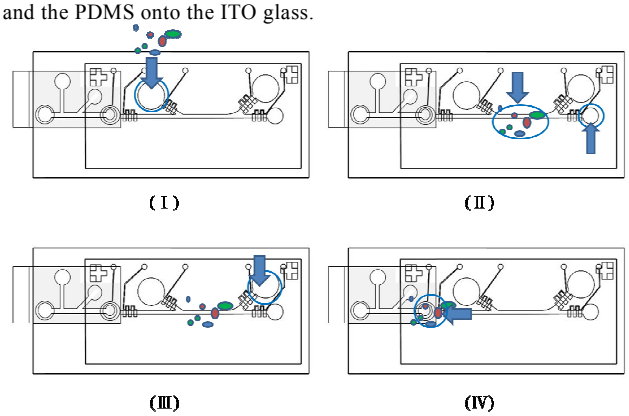


(d)

Figure 1: Device illustration. (a) An exploded view of the different layers showing that the integrated microfluidic device was composed of two modules: an ODEP module and a PDMS microfluidic control module. Two patterned PDMS layers were assembled to form the microfluidic control module. These PDMS layers contained air chambers used to pump liquid in the liquid channels underneath. The liquid channels were used to transport the cells and the different mediums. The top ITO glass was used as the middle substrate for the integrated optofluidic device. Patterned double-sided tape was used to form microchannels for ODEP manipulation between the top ITO glass and the bottom ITO glass coated with an a-Si layer. (b) A top-view schematic of the integrated microfluidic chip with the micro filtering structure. Integrated micropumps, microvalves and micro filtering structures automatically performed the medium replacement process. (c) A SEM photograph of the mcirofiltering structure. (d) A photograph of an assembled integrated microfluidic device. An oxygen plasma treatment was used to bond the PDMS layers together and the PDMS onto the ITO glass.



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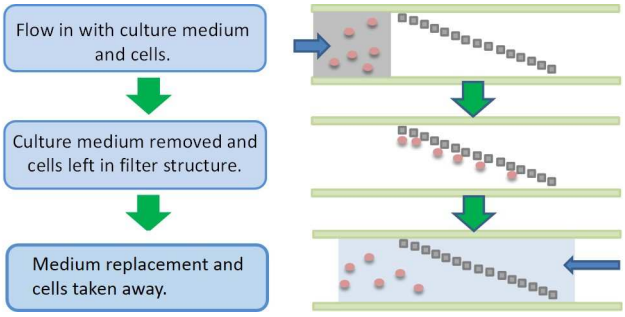


(b)

Figure 2: Cell filtering and medium replacement processes. (a) Illustration of the operating process for the integrated microfluidic device. First, cells (HepG2) suspended in the culture medium were injected into a sample chamber on the PDMS microfluidic control module of the device (I). The cells and the medium were pumped (left to right) by the integrated micropumps onto the micro filtering structure. There, the cells were collected by the filter and the culture medium continued to flow through the filter, thus achieving cell separation (II). Then a 0.2 M sucrose solution was injected into the same sample chamber for a period of time for medium replacement. Next, more 0.2 M sucrose solution was pumped from the opposite direction to transport the perfused cells into the ODEP module (III). The cells were then manipulated or separated by optically-projected virtual electrodes and the ODEP force (IV). After ODEP manipulation, cells were extracted and mixed in cell culture medium, and subsequently cultured in bench-top incubators. (b) Cells in the original culture medium were pumped into the micro filtering structure. The size of a live cell is about 15 µm, which is larger than the space between any two square structures but the original medium would be pumped past the square filtering structures from the entrance chamber to a waste chamber Then, the 0.2 M sucrose solution was pumped into the microchannel from the other entrance chamber, that was on the same side as the DMEM waste chamber. The 0.2 M sucrose solution transported the cells, that were trapped by the filter, to the ODEP layer.

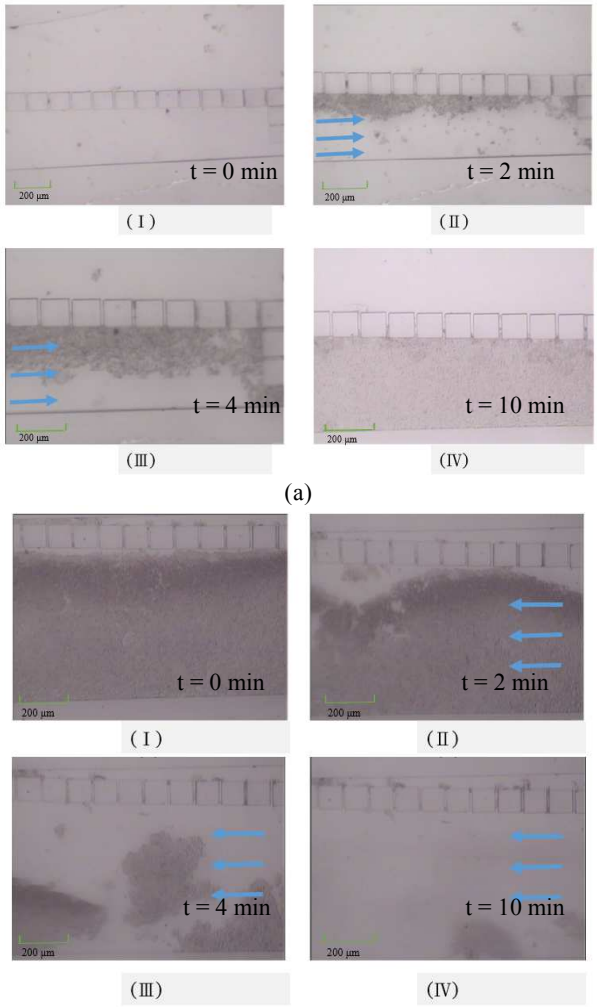
The ODEP force was generated by producing virtual electrodes using a computer, commercial software (FLASH, Adobe, USA) and a digital projector (PJ1172, Viewsonic, Japan). Using this system, we projected a 20 µm wide scanning line onto the ODEP device at an applied alternating-current (AC) voltage of 20 Vpp at a frequency of 100 kHz . Motion was induced in the cells in the 0.2 M sucrose solution when the optical scanning line illuminated the a-Si layer, disrupting the uniformity of the AC electric field. A positive ODEP force was then generated to drag the cells to effectively follow the 20 µm wide scanning line. The generated ODEP force could be measured by the balance of the viscous drag force and the generated ODEP force[15]. Note that live and dead cells experienced different forces such that they could be separated.

3. Results and Discussion

3.1 In-situ medium exchange with live cells

The performance of the micro filtering structure and the in-situ medium exchange process were first characterized. As expected, the micro filtering structure was able to keep the cells in the channel while medium replacement occurred (Figure 3a). Live cells, with the total number of cells ranging from $1x10^3$ to $1x10^5$, were tested and filtered in the channel. This process took only about 10 minutes to collect all the HepG2 cells in the micro filtering structure (Figure 3a). We then injected the 0.2 M sucrose from the same chamber and pumped the sucrose to wash and perfuse the cells for about 5 minutes. We call this the "extra perfusion time", and will discuss its importance later. Then the sucrose-perfused cells were released from the micro filtering structure by pumping additional 0.2 M sucrose solution in the opposite direction via the interconnect leading to the ODEP module. Successful cell trapping and release was observed under the microscope (Figure 3b). Note that the cells were collected manually by using a pipette and the counting was done by a a microscope cell counter (hemocytomer).

The recovery rate of live cells using this automated process was then characterized. Experimental data showed that high initial cell concentrations generally yielded high recovery rates. Up to 91% of the cells loaded into the microfluidic device were successfully recovered when $1x10^5$ cells were tested. However, cells sometimes became stuck in the microfluidic control device, which would lower the recovery rate. The results in Figure 3c suggest that a constant number of cells consistently became stuck in the device, which would explain the decreased recovery rate as the number of input cells decreased. The recovery rate dropped to 68% when only $1x10^3$ cells were tested. Note that these data were from three consecutive measurements with a standard deviation less than 15%.



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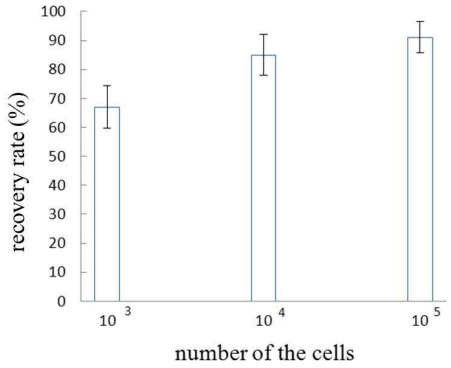


(c)

Figure 3: Cell recovery while the micro filtering structures kept the cells in the channel. (a) A series of pictures (I) to (IV) showing that cells flowing from left to right in the channel were collected by the micro filtering structures. (I) The beginning of medium replacement; (II) Cells collected after 2 minutes; (III) After 4 minutes; (IV) After 10 minutes. (b) A series of pictures (I) to (IV) showing that the collected cells at the micro filtering structures were successfully transported by a flow (from right to left) of 0.2 M sucrose solution to the ODEP layer. (c) The recovery rate of cells after medium exchange with different numbers of cells. The number of cells lost is approximately fixed. Therefore the more cells initially, the higher the resulting recovery rate. Note that all recovery rate experiments were conducted at the same flow rate (~100 µm/s) for all cases.

This study also compared the recovery rate obtained using the traditional method of replacing the medium by centrifugation. The microfluidic control device required only 15 minutes (5 minutes for extra sucrose perfusion and 10 minutes for cell collection) to complete the medium replacement process while the traditional centrifuation method required at least 80 minutes. Although the recovery rate by the centrifugation method was almost 100% (which was only ~9% better than the results obtained using our microfluidic control device), the shorter time for medium replacement ensured a higher cell survival rate. The 0.2 M sucrose solution is an appropriate medium to induce the ODEP force, but not suitable for long-term cell survival. For example, if the total experiment protocol was over 50 minutes, which is a practical amount of time required to perform centrifuge-based medium exchange, the survival rate of the cells would be reduced to about 3% (from our own experiment). Furthermore, the automatic process peformed by the microfluidic control device alleviates the labor-intensive and tedious replacement process.

3.2 Measurement of the ODEP force generated on cells

As mentioned in the previous section, the ODEP force was induced using computer-generated virtual electrodes via a digital projector. The average velocity of the manipulated cells was measured by changing the scanning velocity of the projected optical line and experiments were conducted with respect to the extra perfusion time of the sucrose solution. Note that the extra perfusion time for the replacement (sucrose) medium had an significant influence on the the ODEP force applied on the cells because the concentration of sucrose absorbed by the cells affected its electrical permitivitty and conductivity. Experimental results are shown in Figure 4. Note that these data were from three consecutive measurements with a standard deviation less than 5%. According to the experimental data, the longer the perfusion time, the stronger the resulting ODEP force on the cell. For a five-minute perfusion, the maximum drag velocity was measured to yield 109.1 µm/s. This translates into a drag force of 12.50 pN when calculated using Stokes' law and assuming an average cell diameter of 15 microns[12].

However, this was less than half of the drag velocity obtained when using cells prepared by the traditional method in a centrifuge. Using the traditional medium replacement process, the cell velocity which could be induced by the ODEP force reached up to 266.7 µm/s. This was probably because the traditional method used centrifugation at 1500 rpm for a total of 15 minutes which effectively replaced all the original medium in the cells. Although the ODEP force generated from cells prepared using our device was not as large as those cells prepared using the traditional method, we were still able to use the ODEP force in a constant fluid flow to separate all the live/dead cells within a total experimental time of 25 minutes, which will be demonstrated in the next section.

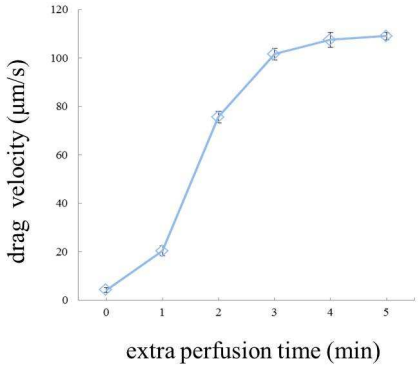


Figure 4: Effect of the extra perfusion time. The maximum drag velocity as cells were dragged by an illuminated line was 109.1 µm/s after 5 minutes of extra perfusion time. The longer the perfusion time, the larger the magnitude of the ODEP force on the cell.

3.3 ODEP separation of live and dead cells

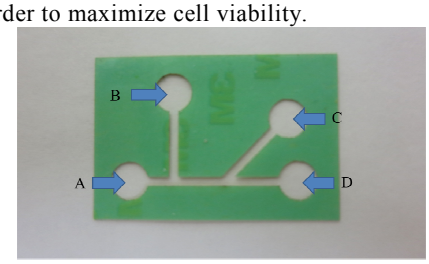
Separation of live cells from dead cells was achieved by injecting cells into the microchannels patterned by double-sided tape, which were connected to four chambers (Figure 5a). Note that the double-sided tape was 30 µm thick which defined the depth of the microchannel. In this chip, one chamber (labeled A in Figure 5a) was for the via interconnect which linked the ODEP module to the PDMS microfluidic module. Another chamber (labeled B) was used as an inlet to provide a focusing flow to squeeze all the cells along the bottom channel. The two other chambers (labeled C and D)

were for collecting the ODEP-separated live and dead cells, respectively. The velocity of the focusing flow was the same as the injected flow so that all the cells were pushed downward to the bottom of the horizontal channel. Note that all the flow velocities of these two flows were set up to be 100 μm/s.

First, all the live and dead cells were driven to the lower chamber D by using the focusing flow from the chamber B without cell separation as a control case. Note that $1x10^5$ cells were tested for the following experiments. Figure 5(II) shows the focusing flow pushing all the live and dead cells to the lower chamber D when the ODEP was turned off. Note that, in this case, 40% of the cells were live and 60% of cells were dead. In these images, dead cells were stained with the Trypan blue dye and live cells were stained with calcein acetoxymethyl ester (CaAM) dyes. Therefore, dead cells appeared as blue (in Figure 5b(II)) while live cells appeared as green when a laser was used to induce fluorescence (Figure 5b(III)). Note that chamber C, where the live cells were collected, would be empty when the flow focused all the live/dead cells to the dead cell collection chamber (chamber D).

For cell sorting, a series of moving light patterns, which generated the ODEP force, were then used to push the live cells to the upper channel towards chamber C[14]. Multiple optical patterns were projected, each composed of parallel lines extending across the width of the channel. The light patterns generated a positive ODEP force that was then used to separate the live cells from the dead cells such that they could be collected into the appropiate chambers. The recovery rate of the live cells was measured to be 96.2% (Figure 5c).

Similarly, the recovery rate of the dead cells was measured to be 74.5% (Figure 5d). This ODEP-based approach allows for the continuous separation of live and dead cells with a higher cell throughput. Although 25.5% of the cells in the "dead cell" collection chamber were still alive, this could be improved by optimizing the flow conditions for more accurate ODEP-based separation in the near future. In our current experiments, we minimized the total processing time in order to maximize cell viability.



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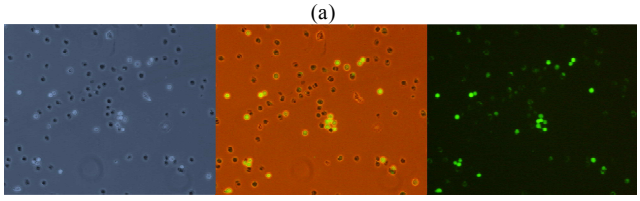

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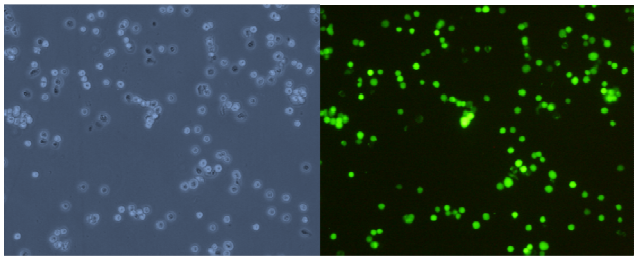

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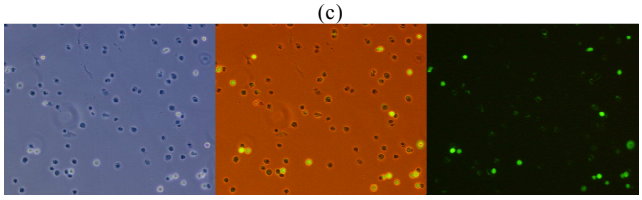

(I) (II) (III)

(d)

Figure 5: Separation of live and dead cells. (a) A photograph of the patterned, 30-μm thick, double-sided tape. Chamber A was the via interconnect to a microchannel in the PDMS layer, and chamber B was an inlet for a focusing flow to squeeze the live and dead cells along the bottom of the horizontal channel. Chambers C and D were used to collect live and dead cells, respectively. (b) These pictures (taken in chamber D) showed that the focusing flow pushed all of the live and dead cells into the chamber D when ODEP was not activated. Note that dead cells were stained with the Trypan blue dye and live cells were stained with calcein acetoxymethyl ester (CaAM) dyes (I). Dead cells appeared as blue in this image (taken in chamber D) (II). Live cells appeared as green when fluorescently induced by laser (image was taken in chamber D) (III). (c) Images taken in the chamber C showed that the number of the dead cells (blue) was greatly reduced when ODEP was used to separate live and dead cells (I). Similary, the number of live cells (green) can be greatly increased using the similar approach (II). The recovery rate from chamber C, which was used to collect the live cells, was measured to be 96.2 %. (d) The recovery rate of the dead cells (blue) (image taken from chamber D) was measured to be 74.5 % (I) when compared with the control case in (b). The image was taken in the chamber C showed that the number of the dead cells was increased (II) while the image taken in the chamber D showed the number of the live cells was significantly decreased.

4. Conclusions

This study demonstrated a new integrated microfluidic chip that performed the entire medium replacement and ODEP manipulation process. The entire medium exchange process was completed in only 15 minutes, which is dramatically faster than conventional, manual, centrifugation processes. More importantly, the survival rate of the cells was greatly enhanced. Furthermore, this new integrated microfluidic chip were able to automatically perform a downstream ODEP-based cell manipulation and separation (for live or dead cells) without manual intervention. The ODEP force in a constant fluid flow could separate all the live/dead cells within 25 minutes. The cell recovery rate, the maximum ODEP force, and the cell survival rate were characterized. Up to 96.2% of the live cells loaded into the device were successfully recovered, thus demonstrating minimal loss due to the fluidic

operations within the device. The ODEP force was able to drag cells at 109.1 μm/s. This strong ODEP force was an indication of the relatively complete replacement of the cell culture medium by the electrically conductive sucrose solution. More importantly, the survival rate of cells was greatly enhanced after all these experiments. By automating the medium exchange and the ODEP-based separation processes, this new device reduced the risk of contamination. This microfluidic chip, therefore, provides a promising platform for the fast replacement of cell medium. This is also the first time that an ODEP device has been integrated with actively-actuated microfluidic devices with automated control.

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