An electrostatic microwell–based biochip for phytoplanktonic cell trapping

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A simple microwell-based microfluidic chip for microalgal cells trapping was fabricated. An electrostatic cell trapping mechanism, enabled by a positively charged glass surface, was used. The chip was capable of capturing multiple algal cell types. In the case of filamentous *Spirulina platensis*, we observed single filament occupancy of up to $\sim 30\%$ available wells, as high as some previously proposed methods. Captured filaments were not of any preferential size, suggesting well randomized cell trapping. It was found that the electrostatic attraction did not affect the cell growth. Total replacement of liquid inside the wells could be achieved by pumping new solutions via the inlet, making single cell experiments in controlled chemical conditions possible. After the top layer of the chip was removed, cells in the wells could be simply transferred using a micropipette, turning the chip into a platform for strain selection. © 2014 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4882196>]

I. INTRODUCTION

Microalgae are a highly diverse group of primitive photosynthetic microorganisms. They are rich sources of a wide spectrum of valuable products such as proteins,^{1,2} pigments,^{2–5} antioxidants,^{2,6} fatty acids,^{2,7} and pharmaceutical compounds.^{2,6} Recently, algae have gained even more attention from scientists around the world as food and alternative energy crops. Their ability to thrive in various harsh culturing conditions and high growth rates⁸ make algae attractive candidates for foods and alternative energy production.

The key to a successful algae-based industry is fundamental knowledge of algal cell behaviors, growth and their interaction with the environmental factors. Most understanding of algal biology has been based on descriptive observations or conventional quantitative measurements on samples collected from bulk scale culture (optical density, sample cell count). One example is the work by Cifferi⁹ in which a descriptive *Spirulina platensis* life cycle was redrawn and is still in use of today. Scientists started to realize these methods are not sufficient to truly understand the algal cells, as the measurements assume individuals in the population of the culture behave exactly same.^{10–12} Observation of diversity of characters and capabilities of the true population is hindered by the data acquisition. Unlike adherent cell types such as mammalian cells, living phytoplanktonic cells, especially over a long period of time, has been poorly

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studied. The major barrier is the fact that the cells are not only microscopic but also planktonic—being free floating in the culture medium. Investigation of the algal cell under a controlled environment has been technically problematic.

In the past few years, there have been several proposed new techniques from many groups to overcome the problems of trapping different species of cells, including algae, on microfluidic chips. These techniques not only allow several single cells/colonies/filaments to be tracked for quantifying individual growth over a long period of time but also enable quantitative observation of the algal cell behavior continuously in a culture. However, some technical limits still remain.

One of the most famous techniques is the microdroplet-based cell encapsulation in oil phase^{10,11,13} separating cells into private compartments. It facilitates the rapid tracking of single cells in parallel but only in a static culture mode. An addition or removal of chemicals cannot be simply made. Trapping of cells with an electric field, using electrodes,^{14–16} may allow cells to be captured in place under regular medium replacement. The technique, however, is not very practical for repeated biological routines and is not very cost-effective. The electrical field may also affect cell proliferation.^{17,18} Cell trapping based on suction,^{15,19} channel constriction²⁰ or microcage²¹ may be considered a less costly alternative. Their great difficulty is that there is not a single universal trap design of this kind that can handle multiple types of algal species. The algal cells come in a great variety of shapes and sizes. A tailor-made mechanism is needed for work with each specific cell types. Long-term cultivation can be even more problematic as the trap has to be capable of keeping the whole population of daughter cells in place. A design based on channels with a closed end has been successfully used in studying cell wall growth²² and tracking lineage²³ of rod-like bacteria. However, the algal cell morphology is diverse. The channel-based design is not applicable to algae. So far, there has not been a simple versatile single trapping mechanism capable of use with a board range of algal cell types and allowing truly flexible user-defined chemical control during the experiment. Experiments under continuous culture or adding gradients of growth factors or inhibitors have not been possible.

Microwell-based trapping mechanisms have been acceptably used in capturing a wide range of cells such as mammalian cells^{24–26} and yeasts.^{27,28} The intuitive nature of the well structure is that it allows any particles that are smaller than its size to fall within non-specifically. The wells' depth also acts as a barrier to prevent particles fallen into the well from being easily lifted away when there is a liquid flow above the well. Furthermore, a well structure is also relatively easy to construct repetitively with conventional microfabrication technology. However, it has not yet been designed for use with algae. This is probably because many microalgae are non-adherent, and after settling, may freely float away from the wells, dependent upon the cell status and light.^{29,30}

In this paper, we propose a biochip design that uses the advantages of a microwell as an economical and simple cell capturing mechanism for studying the growth and behavior of single planktonic microalgal cells in a controllable medium flow in which gradients of chemicals (i.e., nutrients, growth factors, and growth inhibitors) can be created or removed at any users desire. To overcome the problem of the cells floating away, an array of microwells was constructed using a commercial positively charged glass slide as the base, facilitating an electrostatic field to trap the planktonic cells inside the well for long run single cell observation under a continuous flow. An economically important algae, *Spirulina platensis*, was chosen as the major organism model in the study because it exhibits a highly diverse multicellular filament's length (from a few microns to several hundred microns). Optimization of the cell seeding condition, to reach high single cell trapping, was conducted. Validity of the device was demonstrated through different studies such as chemical perfusion within the wells, size distribution of cells captured in the device and growth kinetics of cells in the device.

II. MATERIALS AND METHODS

A. Design, fabrication, and characterization of the biochip

The chip is a microscope slide-size (75×25 mm) cell chamber containing an array of 200 microwells (width $\times$ length $\times$ depth = $1000 \times 1000 \times 500$ μm) in the base. This size fits the

large algal cells used, which were in the range of a few hundred microns in diameter. The array contains 10 rows of 20 wells with $500\ \mu\text{m}$ spacing. The wells were formed by plasma bonding a $500\ \mu\text{m}$ thick polydimethylsiloxane (PDMS) layer with a pattern of the bottomless microwells to a commercially available positively charged microscope slide (SuperFrost® Plus J1800AMNZ, MENZEL-GLÄSER, USA). This was then covered by a diamond shape fluidic layer. See Fig. 1(a). Fig. 1(b) displays a magnified image of the connection between the inlet/outlet to the chamber. The tip of the silicone tube was embedded to the ceiling so its opening end was exposed to the fluidic space.

The microwell layer was created by standard PDMS soft lithography using the nickel mold which was fabricated using X-ray LIGA technique³¹ (Fig. 1(c)). The technique was suitable for fabrication of a “tall” micropattern with a microscopically smooth surface. The metal was used because it is a good material choice of permanent prototype mold for repeat casting of PDMS. Alternatively, a heat resistant resin could be used.

The pre-polymerized PDMS was poured on the flat area of the mold and then spread to the micropatterned area. Like this the thickness of the microwell can be adjusted by changing the volume of the poured PDMS. A thickness of $\sim 100\ \mu\text{m}$ was achieved.

On top of the microwell layer is a fluidic layer. It has a flat top with a diamond shaped flow channel on the reverse side. The depth of the channel determines the height of the cell chamber, which is about 1 mm. The specified dimension of the fluidic layer is shown in Fig. 1(d). The inlet and outlet are connected to the ends of the channel allowing the flow of liquid media. This layer was made by pouring pre-polymerized PDMS on a laser cut acrylic mold

FIG. 1. The fabricated device. The device is composed of 3 layers: the positively charged glass slide, microwell layer, and fluidic layer (a); detail of the structure of the connection between the tubing and inlet/outlet is displayed. Tube is embedded into the volume of PDMS so the tube end is connected to the fluidic space (b); the microwell layer was fabricated using a nickel mold produced with X-ray LIGA technique (c); dimension of the fluidic layer as seen from the top view is specified (d); cross-section (e) and picture of the finished device (f) are displayed.

that had a rectangular frame and diamond shaped island at the center. The diamond shape was thinner than the frame so it gave the embossed patterned of the channel to the finished layer. Tubing and contacts between layers of the device were sealed using pre-polymerized PDMS followed by heating. Figs. 1(e) and 1(f) display the cross-section of the device and the finished device, respectively.

To ensure the expected outcome of the micro-patterned structure, the depth of the micro-wells (the thickness of the patterned layer), was measured from 9 different area of 3 fabricated arrays, using a digital micrometer (Mitutoyo, Japan). The structure of the wells was investigated using an inverted microscope (Nikon TS100F). Digital micrographs of the wells were captured under the inverted microscope with a digital single-lens reflex (DSLR) camera (Canon D110) through its microscope adaptor. The width of the well was measured from digital micrographs of 28 different wells randomly selected from 4 arrays using the freeware, ImageJ.³²

B. Algal strains and culture

Algal strains used were *Spirulina platensis* strain CMU 2, *Pediastrum* sp., *Ankistrodesmus* sp., *Chlorella vulgaris*, and *Scenedesmus* sp. obtained from Applied Algal Research Unit, Department of Biology, Faculty of Science, Chiang Mai University, Thailand. All the stains were maintained in BG-11 medium³³ except *S. platensis*, which was cultured in Zarrouk's medium.³⁴ Preparation of these standard algal culture media are described elsewhere.^{33,34} *S. platensis* were cultured at 30 °C under 4k lux fluorescent illumination and rotary shaking at 120 rpm (SHO-2D, Wisd Laboratory Instruments, DAIHAN Scientific Co., Ltd., Korea).

C. Cell loading and cultivation in the chip

Prior to the cell loading, the fluidic channel was subsequently rinsed with 70% ethanol, sterile distilled water and sterile algal cell medium. After rinsing, the chip was fully filled with sterile medium before cells infusion. To load the cell to the biochip, the algal cell suspension in the appropriate medium was filled in a plastic sterile syringe. The cells suspension was then injected using a syringe pump (NE-1000, New Era Pump Systems Inc., USA) at a constant flow rate of 200 $\mu\text{l}/\text{min}$. This particular flow rate performed effectively in our preliminary experiment with *S. platensis*, and thus, the experiments and optimization in this study were done based on this rate. At the end, another round of sterile medium was injected into the device to purge the remaining uncaptured cells.

In our further cell loading optimization with *S. platensis*, different combinations of filament concentration in the suspension (1000, 2500 or 5000 filaments/ml) and volumes of the algal suspension, making up a total filament number of either 500 or 1000 filaments, were loaded into the device at a constant flow rate of 200 $\mu\text{l}/\text{min}$. For instance, in the case of 1000 filament/ml suspension, 500 μl and 1000 μl of this suspension was prepared and infused so that the total number of 500 and 1000 filaments was loaded into the device. The algae cultured in the biochip were kept in the same lighting and temperature setting previously described in the cultivation section.

D. Imaging of cells and analysis

In the study of the cells' growth kinetics in the biochip, the algal cells were observed and counted to monitor their growth under an inverted microscope (Nikon TS100F). Digital micrographs of the cells were captured under the inverted microscope with a DSLR camera (Canon D110) through its microscope adaptor. Measurement of cells length in the micrograph was done manually using the measuring tool of the freeware, ImageJ.³²

E. Chemical perfusion inside the biochip and analysis

The biochip was first filled with either Zarrouk's medium (blank) or 1 mM Azorubine in Zarrouk's medium (dye solution). Then, the other solution, i.e., the dye (adding mode) or blank medium (washing mode), was injected into the device to replace the first solution at a constant

flow rate of either 200 or 400 $\mu\text{l}/\text{min}$. The picture of the wells (around the center of the array, $n = 4$) was captured every 50 s or more frequently under the inverted microscope with attached DSLR camera in manual mode. The intensity in the red channel of the well area could be measured using ImageJ. The intensity values were converted into a local concentration using a calibration curve constructed from a set of images taken from wells filled with dye solutions of known concentration. The relative local intensity (100x local intensity/the local intensity when the device filled with the blank) was plotted against time.

III. RESULTS AND DISCUSSION

A. Biochip characterization

The obtained microwell layer of the biochip was investigated using the micrometer and optical microscope. The depth and width of the well measured were $523.7 \pm 27.9 \mu\text{m}$ (mean $\pm$ SD, $n = 3$) and $1067 \pm 24 \mu\text{m}$ (mean $\pm$ SD, $n = 28$), respectively, similar to expected dimension. The square shape and a fairly straight edge of the wells were observed, see Fig. 2. This indicated that the array of microwell can be obtained using the novel protocol. The soft lithographic procedure using the resin mold, an economical substitution of the metal mold, produced a similar result. The simplicity of the protocol and reproducibility of mold at low cost heightens the potential of the biochip to be constructed and used in any biological laboratory with minimal technical prerequisites.

B. Cell capturing

All the algal cells including *Pediastrum* sp., *Ankistrodesmus* sp., *Chlorella vulgaris*, *Spirulina platensis*, and *Scenedesmus* sp. (Fig. 3) could be successfully captured in the biochip. By increasing the speed of medium injection to the device up to 900 $\mu\text{l}/\text{min}$, no cell detachment was observed. This suggests a sufficiently strong capturing ability of the well under a continuous stream of culture medium.

In contrast, cells were not captured in the wells when the positively charged microscope slide was replaced with an uncharged one. Cells were flushed away after the final medium

FIG. 2. Displays the stitched micrograph of the micro-patterned layer. The image suggests that the desired rectangular well structures were successfully formed by the developed fabrication process. The scale bar represents ~ 1 mm.

FIG. 3. Algae from different taxonomic groups could be captured by the device. Example of trapped algae displayed here are *Spirulina platensis* (a), *Chlorella vulgaris* (b), and *Scenedesmus* sp. (c). Pictures of magnified groups of trapped cells are presented in (b) and (c). The yellows and black scale bars represent 100 and 10 μm , respectively.

injection. This indicated that the capturing in the biochip occurred via electrostatic interaction between the positively charged glass slide and the negatively charged cells surface as expected.

It was found that the species of smaller cell size such as *C. vulgaris* and *Scenedesmus* sp. tended to be trapped more densely in single wells, even when the same cell concentration was used (Figs. 3(b) and 3(c)). Further optimization of cell loading conditions may be required for each species. In addition, the size of the wells may be adjusted to fit smaller cell types. However, these experiments suggested that this electrostatic microwell can be used in capturing planktonic algal cells of a diverse shape, size, and taxonomic group.

In our further cell loading optimization with *S. platensis*, a total number of either 500 or 1000 filaments, were loaded to the device at a constant flow rate of 200 $\mu\text{l}/\text{min}$ ($n = 3$ each). The cell number used for loading affected the number of cells trapped in the well. The percentage of wells found trapping different numbers of *S. platensis* filaments are displayed (Fig. 4). It can be obviously seen in the graphs that the majority of wells did not trap cells. The percentage of wells containing cells decreased with the number of cells the well trapped. This percentage decaying trend was found in all optimization experiment.

However, the major concern is the percentage of wells containing a single cell. The larger this percentage, the more repeat experiments with single cells that can be performed in a single set up. In general, the percentage of single cell occupancy varied within the range of $\sim 10\%$ – 30% . The highest percentage of 30.3 ± 1.5 (mean $\pm$ SD) was achieved when 500 filaments were loaded using a 2500 filament/ml cell suspension (Fig. 5).

Fig. 6 depicts distribution of cells captured in different location of the biochip when different number of cells loaded using cell suspension of various concentrations. In most cases, captured cells were found to be equally distributed within the array. The clear patterns of preferential cell accumulation towards the inlet were found when the suspension with the lowest cell concentration (1000 cell/ml) was used. In addition, a large proportion of wells under this condition, especially those near the inlet, also captured more than 1 filament.

At lower cell concentrations, greater volume of cell suspension for the targeted number of cells infused was required to flow through the device, and therefore, the more time the wells were exposed to the cell suspension. In addition, for a given time period, most of the cells were captured by the wells located at the front (near inlet) and even less remained for the subsequent wells. This

FIG. 4. Percentage of wells containing trapped cells of different numbers when different total cell numbers and cell concentrations were loaded to the device at the constant speed. The percentage was calculated based on how the cells occupied in the well in the biochip. The bars represent the average of the percentage with the error bar displaying the SD; the values based on triplicate results. The trends of the percentage in all the experiments follow the same decaying trend with the highest frequency of the empty wells. The total cell occupancy in one single well could be as high as 12 cells per well.

could explain the disproportionate accumulation of cells towards the inlet when lower cell concentration was used.

Infusion with highly concentrated cells suspension reduced severity of the bias captured cell distribution. It is also intuitive that the success rate of capturing should depend on cell

FIG. 5. Percentage of wells containing single cells trapped when total filament numbers of 500 or 1000 filament were loaded using different suspension of different cell concentration (1000–5000 cells (or filaments)/ml). The bars with the error bar represent the means with their SD based on 3 different repeat experiments ($n=3$). The highest percentage of single cells trapped in a single well reached 30.3 ± 1.5 when 500 cells were loaded using the cell suspension with a cell concentration of 2500 cells/ml with the constant flow rate.

FIG. 6. Distribution map of trapped cells in the biochip when different total cell numbers (500 or 1000 cells) were added to the chip using different concentrations of cell suspension (1000–5000 cell/ml). 6 tables contain 10×20 cells represent the microwells presented in the chip. Well columns on the most left is the closest to the inlet whereas the most right is the nearest to the outlet. The cell colored in yellow and red represent occupied well with number of cell = 1 and >1 cell, respectively. Numbers of cells captured are also specified. White cells are empty wells.

concentration; the denser the cells, the more likely that at least one cell get captured. However, according to our experiment, it resulted in slight to moderate drop in cell capturing efficiency. This could be due to the fact that less suspension volume was required to be flowed through the chip to reach a certain loaded cell, in comparison to the case of lower cell concentrations. As attraction of suspended cells to the trap is a probabilistic (random) event, beside the density, the chances of cells being capture may also be a function of time. In other words, the longer the well exposed to the flow of suspension, the more likely the cell capture (at least one cell) will be successful.

Therefore, the capturing efficiency in this biochip was a balance between cell concentrations and time the wells exposed to the cell suspension. Either of these two extremes resulted in reduction of the capturing efficiency. This implies why the capturing efficiency, in terms of single cell capturing, number of wells occupied and non-bias cell accumulation, reached the highest when 2500 cells/ml cell suspension was used, among others in this experiment.

Another factor involved in capturing efficiency was the total number of cells loaded. It was also another factor determining how long the wells are exposed to the stream of suspended cells. Expectedly, it was found that doubling the number of total cell loaded resulted in larger number of cells trapped, globally. Unlike the pair of “time” and “cell concentration,” in the investigation of cell concentration, the total cell number did not change the preference of cell accumulation but the frequency of cell found. As seen in Fig. 6, patterns of cell accumulation in the experiments with same cell concentration were similar but intensified when cell number increased.

The highest efficiency of single algal cell trapping was equivalent to those in some previous mechanisms used in other works.^{10,11} This means that about single 60 *S. platensis* filaments will be provided in a single chip, which is sufficient for a broad range of experiments such as tracking growth kinetics of the filaments, cell-environmental interaction and cell screening.

C. Preference of cell size trapped

It is necessary to ensure that the trapped filaments represent the actual population present in the bulk culture (i.e., cell cultured in an Erlenmeyer flask). *S. platensis* exhibit a wide range of filaments length distributions in a given culture (a few tens of μm to a few hundreds of μm). As size is closely related to cell maturation and physiology, we wondered if filaments of particular sizes (i.e., smaller) may be more likely to be trapped, causing a selective sampling. To answer this question, we took microscopic images and measured the length of 200 trapped filaments in the biochip using imageJ. The same experiments were conducted using algae from 3 different days of culture: day1, day2, and day5. These algal cells were cultured in $\sim 150\text{ml}$ culture volume in Erlenmeyer flask. The length distribution of the filaments from each day was constructed based on the frequency found. This was then compared to that of the algal population directly sampled from the bulk culture of the same day.

It was found that all the length distributions fit a similar peak-like pattern (Figs. 7(a)–7(c)). Diversion of the peak characteristics such as the maxima, the x-axis value at which the maxima occurred, were observed upon day of algal collection. Although the distribution changed with day of culture, a similar pattern of distribution was found between the trapped cells and the cells in bulk culture on the same day. This means that cells of a given single species trapped by the biochip are a well randomized sample representing their population in their bulk culture.

D. Effect of the electrostatic charged to cell growth kinetics

The employment of a positively charged surface to capture the cells may be considered controversial. Similarly to trapping cells with an electric field using a micropatterned electrode, electrical charge may affect the cells.^{17,18} It may also be of a concern that preventing cells to

FIG. 7. Length distribution of the *S. platensis* filaments found captured in the device in comparison with that in the bulk culture. The experiment conducted using algal samples from 3 different days of cultivation, the distribution of the captured cells and cells in the bulk are similar in all the experiments. Each distribution was based on 200 filaments counted. The trend lines display the data sets fitted to Gaussian distribution ($y = a \times \exp(-0.5 \times ((x-b)/c)^2)$; when a , b , and c are regression coefficients).

be naturally free-floating eventually results in changed growth kinetics. To study the effect of the charged surface on growth kinetics, we monitored groups of *S. platensis* filaments grown under 3 different conditions (10 groups each, mean $\pm$ SD of initial filament number = 9.8 ± 1.4): (1) in wells with the positively charged based, (2) in wells with normal glass surface base, and (3) in static bulk culture (500 ml flask).

As earlier mentioned, cells could not be trapped in the biochip without assistance of the positively charged microscope slide. Therefore, the conditions (1) and (2) were conducted in a similar biochip but with a larger area of circular wells (5 mm in diameter and 1 mm in depth). The PDMS fluidic layer was removed. 20 μ l initial cell suspension was directly added to the wells with a micropipette. Another piece of microscope slide was put over the well layer with the help of a 2 mm thick spacer. This caused a 1 mm gap between the wells and the ceiling that minimized the evaporation of the medium and also avoided a direct contact which could disturb the liquid in the well. The whole biochip was kept in a moist chamber to prevent medium drying. The cells number in the wells was directly counted from the well slide. To count cells in the bulk culture (condition 3)), a 20 μ l cell suspension was pipetted from the flask and dropped on a microscope slide before counting under a microscope.

It was found that cells under the 3 conditions grew in a similar pattern (Fig. 8). They entered the logarithmic and stationary phase of growth on the same day and had a very similar specific growth rate (μ) during the logarithmic phase. Calculation of μ was done using the following equation:¹⁰

$$\mu = \frac{d(\ln N)}{dt},$$

where N is number of filaments and t is time. Results were in agreement with the previous finding indicating that growing algae in a relatively small scale volume does not alter the cells' growth kinetics.¹⁰ Based on the same previous study, it should be noted that this is only true for the case of a small population of multiple cells. Kinetics of single cell growth varies enormously which is thought to be due to individual physiological differences and the stage of cell cycle each individual is at.¹⁰ This was the reason our experiment started with an initial filament number of about 10.

Another interesting aspect of our result is that the deviation of the average filament number during different days of experiments was very low. This was probably because a larger number of repeat experiments were conducted ($n = 10$) in comparison to previous studies.¹⁰ In addition, unlike the previous study¹⁰ in which single cells of *C. vulgaris* were the model organism, the multicellular *S. platensis* were studied. Ten filaments of *S. platensis* may contain hundreds of

FIG. 8. The growth curves of *S. platensis* in 3 culturing conditions: in bulk culture, in a well with a normal glass bottom, in a well with the positively charged glass bottom. The scatters represent mean of filament density from 10 observed groups ($n = 10$). The error bars are SD of the means. Cells in all conditions entered different phases of growth on the same day and own a similar maximum specific growth rate of 0.967 ± 0.001 , 0.967 ± 0.001 , and 0.966 ± 0.001 , respectively.

FIG. 9. Exponential relationship between the dye concentration and dye intensity in the red channel measured from the digital photographs ($n=4$), error bars = ± 1 standard deviation. The relationship fit the equation $y = 60.4 + 131.7 \times \exp(-2.6x)$ with R^2 of 0.99.

cells, which sufficiently evens out the physiological divergence among the micropopulation in any single experiment.

E. Chemical perfusion in the microenvironment

This chip is designed to allow chemical environment to be tunable by the user. It is thus essential to ensure that the added chemicals completely perfuse the culturing microenvironment.

Prior to the experiment, the light intensity-dye concentration relationship was constructed as shown in Fig. 9. The relationship fitted well an exponential equation $y = 60.4 + 131.7 \times \exp(-2.6x)$ with R^2 of 0.99. For any local dye intensity measured from the digital photograph of the well taken from the control light setting, the local concentration can be calculated.

From the washing and adding dye experiment, we found that total perfusion in the “adding” and “washing” mode occurred within a time frame of not longer than 500 s but that saturation was reached at different times, depending on the flow rate (Fig. 10). The higher flow rate meant the quicker replacement. In general, it took ~ 300 s and ~ 550 s to get the wells 80% and 100% “washed,” and ~ 100 –250 s and ~ 250 –500 s to get the wells 80% and 100% “added,” respectively. The magnitude of the time taken for the initial chemical to be fully replaced (hundreds of second) is acceptable when considering to the alga’s doubling time, which is about one day.

FIG. 10. Replacement of solution in the device at different speeds of flow. The data points represent mean relative local concentration of dye in four different wells ($n=4$), error bars = ± 1 standard deviation. (a) displays the replacement of the blank medium with dye solution whereas (b) represents the replacement of the dye solution with the blank.

Although the initial relative concentration started at 100%, in one of the experiment (i.e., washing at 200 $\mu\text{l}/\text{min}$) the dye concentration reached nearly 120% within the first minute of the washing. It is possible that this could happen as a result of a transition of the liquid: from being static to being moved. The dye solution was filled and remained static until the constant flow of the blank was introduced. The “push” generated by introduction of the blank caused a temporary compression at the beginning as a result of inertia. This compressed dye solution could temporarily undergo refraction index change and appeared as a spike in optical density, and thus, concentration.

An interesting flow phenomena observed, when “the second solution” was being introduced to the chip, was that the observable and almost linear boundary between the first and the second solution was formed. This boundary moved towards the outlet and took about 5 min to reach the other side of the chip. This indicated the infusion took place primarily through laminar mode of flow of the second solution. Second, as the wells are identical and arranged in symmetrical rectangular array, they should experience the flow in similar fashion. However, with the flow rate used, it took about 2–3 min for the linear boundary of dye to travel from the first column of wells to the last. This caused the “lag phase,” the phase before the leaping rise or fall of the relative concentration in the perfusion kinetics in Fig. 10. The leaping phase started coincidentally with an arrival of the linear boundary. This suggests that, although the perfusion phenomena in all wells should be of a similar fashion, the variation in lag phase can be expected in wells located at different distances from the inlet.

As noted that the perfusion experiments were done based on wells selected from the middle of the array (approximately column 9–11 from the inlet and a row 4–6 from either long edge of the chip). The perfusion phenomena may not absolutely represent the exact perfusion kinetics of wells all across the chip. It describes a generalized idea about what can be expected from the infusion using the middle area of the chip as an average. With known distance of the wells’ locations to the inlet, the delayed perfusion can be adequately estimated. However, the delay should be within 2–3 min which is negligible when considering cell doubling time. In addition, from the knowledge above, it can ensure that there is no “dead zone” in the biochip and the biochip can be thoroughly perfused within the relevant time frame.

IV. CONCLUSION

We successfully fabricated a biochip with electrostatically attracting microwells for planktonic cell trapping. It was demonstrated that this kind of trapping mechanism is capable of trapping multiple planktonic algal cells of different species. The trapping is strong enough to hold the cells in the well under a flow rate of up to 900 $\mu\text{l}/\text{min}$. By adjusting the number of total algal filaments and concentration, the highest occupancy of single *S. platensis* filament reached in the biochip was about 30%. The algal filaments found trapped by the devices are a well-randomized sample of the population they represent in the bulk culture. Further study may focus on the effects of the flow rate and geometry of the well in determining capturing efficiency of algal cells of diverse morphology. Cells grown in the electrostatic microwells exhibited the same growth pattern as those grown in non-electrostatic wells and in the bulk culture. This implies that the electrostatic mechanism can be used to immobilizing cells without altering the cell growth pattern. Chemicals in the culturing environment can be fully replaced within a physiologically acceptable time frame. Faster replacement is possible by increasing the flow speed up to at least 900 $\mu\text{l}/\text{min}$.

Beside its efficiency, the apparent advantages of this design are its simplicity and versatility. The chip could be fabricated and operated with minimal technical knowledge. Unlike some of the previous designs in which trapped cells could, to some extent, freely float or move in 3D space, electrostatic field immobilized cells to the bottom. This is ideal for qualitative and quantitative imaging. Wells organized symmetrically in a rectangular array made well numbering straightforward both automatically and manually. Another separate piece of microscope slide (75 $\times$ 25 mm) was fabricated with a pattern of numbers corresponding to the location of wells. This made a manual search of particular wells under microscope easier, especially for a long

term monitoring of cells in multiple wells. Addition or removal of gradients of chemicals can be added to the chip via the inlet both automatically and manually at any time of experiment. The design fits a wide variety of experimental set up such as observation on temperature-controlled microscope stage, in an incubator, or even in a water bath. The possibility of using heat resistant resin, an economical alternative to the metal mold, increases the practicality for any biological laboratory.

Unlike other algal cell trapping chips, another feature of this device is that it allows users to simply transfer the cell from any particular well to another location. We found the top layer of the device can be simply lifted off exposing the microwell layer. The algal cells remained attached afterwards. By using micropipette, we successfully picked up the cell samples directly from wells of interest. This makes this biochip usable as a platform for the testing and screening of phytoplanktonic strains.

Conventionally, testing and screening of phytoplankton was done by cell isolation using microbiological methods, such as streak plate or cell picking using a capillary tube, before upscaling the initial cells into a mass culture. Clearly, these procedures are time- and resource-consuming and laborious. In contrast, with this device up to 60 single cells at a time can be simply isolated in the wells. After observing the characteristics of each of the isolates under the user-defined culturing conditions, desirable cells can be manually transferred for mass culturing. This makes the biochip not only a simple versatile platform for fundamental experiments but also for testing and screening algal strains for biotechnological purposes.

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