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FORMING SELF-ASSEMBLED CELL ARRAYS AND MEASURING THE OXYGEN CONSUMPTION RATE OF A SINGLE LIVE CELL

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Abstract

We report a method for forming arrays of live single cells on a chip using polymer micro-traps made of SU8. We have studied the toxicity of the microfabricated structures and the associated environment for two cell lines. We also report a method for measuring the oxygen consumption rate of a single cell using optical interrogation of molecular oxygen sensors placed in micromachined micro-wells by temporarily sealing the cells in the micro-traps. The new techniques presented here add to the collection of tools available for performing “single-cell” biology. A single-cell self-assembly yield of 61% was achieved with oxygen draw down rates of 0.83, 0.82, and 0.71 fmol/minute on three isolated live A549 cells.

Keywords

Single-cell; oxygen measurement; microfluidics; self-assembly; and SU8 toxicity

INTRODUCTION

The Microscale Life Sciences Center (MLSC) at the University of Washington is dedicated to performing multi-parameter analysis at the single-cell level [1]. Studying individual cells allows for a more comprehensive understanding of a cell’s activities. When cells are studied in large populations, irregular responses from so-called outlier cells can go undetected due to averaging of the data. Understanding the characteristics of outlier cells is believed to be essential for developing better diagnoses and treatments of such ailments as heart disease, stroke, and cancer.

Oxygen was the first parameter chosen to be measured in our study due to its importance in general cell health and metabolism. While difficult to measure, the instrumentation required to detect oxygen consumption on a single-cell scale set the stage for detection of other molecules of interest to biologists. The MLSC has developed a system that is capable of measuring the oxygen consumption rate of a single cell with fmol/minute resolution [2]. The measurement is performed by sealing individual cells in a microchamber and optically probing a Pt-porphyrin sensor located inside the micromachined micro-wells. The first step in this process is to individually locate live cells on a substrate in order for them to attach

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and later be sealed and measure the oxygen concentration inside the micro-wells. Repeating this measurement over time provides the oxygen consumption rate of individual cells. Segregating and localizing the cells in a high-yield manner have shown to be a major obstacle in the measurement technique. In order to study the population outliers, data needs to be collected on tens of thousands of individual cells. Without an efficient locating method, gathering such a large amount of data would be an extremely tedious task.

In this paper we present a new method of locating individual cells on to a glass substrate using microfluidics and microfabricated structures. A toxicity assessment was performed in order to determine the compatibility of the patternable polymer with the cells. Additionally, a representative experiment was performed showing the oxygen consumption rates of single live cells.

MATERIALS AND METHODS

Cell Trapping

In order to trap the cells, SU8 (SU8-25, MicroChem, Newton, MA) was photolithographically patterned onto borosilicate glass (D-263, Erie Scientific, Portsmouth, NH) in a cup-like shape, 35 μm tall spaced 300 μm apart center-to-center (Figure 1). Before self-assembly, a flat 4-by-4 mm glass ceiling was placed on top of the SU8 structures to create a microchannel for the cells to flow through. The glass ceiling was supported by SU8 support walls that were the same height as the traps. Epithelial lung cancer cells (A549, American Type Culture Collection, Manassas, VA) were used to test the single-cell self-assembly method. The medium used consisted of Dulbecco's Modified Eagle Medium (DMEM) with 4,500 mg/L glucose, L-glutamine, and sodium pyruvate (Invitrogen, Carlsbad, CA) with 5% FetalClone III (HyClone, Logan, UT). Medium containing A549 cells was then injected at the entrance of the microchannel using a syringe pump (NE-1000, New Era Pump Systems, Inc., Wantagh, NY) to maintain a constant fluid flow. Cells were caught in the traps, driven by gravitational and fluidic forces (Figure 2). Each micro-trap group consisted of 9 sites.

SU8 Toxicity

In order to determine the biocompatibility of the SU8 micro-traps with the cells of interest, a systematic toxicity test was performed. The test involved two different cell lines and was conducted over four days. One Barrett's esophagus precancerous cell line was chosen (CP-DhTRT, Fred Hutchinson Cancer Research Center, Seattle, WA) and the A549 epithelial lung cancer cell line. The A549 cells were cultured in the DMEM solution previously described while the CP-DhTRT cells were cultured in Keratinocyte-SFM (Invitrogen, Carlsbad, CA) with 1% FetaClone III.

Three different 15-by-15 mm glass chips were fabricated to use in the experiment: chips that contained 1) no SU8, 2) flat SU8 covering the whole surface, and 3) patterned SU8. The flat SU8 and the patterned SU8 were fabricated following the manufacturer's recommended process with the exception that a mask was not used for the flat SU8. The flat SU8 chip type was included in the experiment to compare any toxicity differences between a large and small amount of SU8 exposure and development.

Each cell line was seeded onto two of each type of chip. The cells were examined two and three days after seeding. Before each set of images, the cells were stained with two fluorescent dyes: 5 $\mu\text{L/mL}$ of 2.5 μM cell viability stain Calcein AM DNA binding dye and 2 $\mu\text{L/mL}$ of 500 μM of cell death indicator SYTOX Orange cell impermeable dye (Invitrogen, Carlsbad, CA). Separate chips were used for each day in order to prevent any contamination resulting from the dyes. The chips were imaged using a fast acting camera

(Andor, South Windsor, CT) connected to an inverted Axiovert 200M microscope (Zeiss, Thornwood, NY). Once the images were collected, they were processed with in-house cell counting software.

Oxygen Measurement

Once cells were trapped, the entire self-assembly setup was submerged upright in medium and cells were left to attach overnight at 37°C in a 5% CO₂ incubator. The ceiling used during self-assembly was then removed. The cells were next stained with the same dyes previously described to verify vitality and sealed using inverted microwells containing sensor that aligned over each cell micro-trap (Figure 3).

The glass ceiling was fabricated by a chrome lift-off step using conventional photolithography followed by etching microwells into the glass using hydrofluoric acid. The chrome was then removed to result in a 3-by-3 array of 115 µm diameter and 45 µm deep wells spaced 300 µm apart center-to-center. An oxygen sensor in the form of Pt porphyrin polystyrene beads (Invitrogen, Carlsbad, CA) was deposited into each well and slightly melted to ensure adhesion using previously developed methods [3].

Absorption of radiation from the light source (405nm Diode Laser, Power Technology Inc., Little Rock, AR) excites the sensor molecule embedded in the polymer beads to an excited state. The molecule could emit light as it relaxes to the ground state. Instead of this radiative energy relaxation route, it is possible for the excited molecule to exchange energy with molecular oxygen. The second non-radiative energy transfer route shortens the effective lifetime of phosphorescence emission from the molecule and is affected by the number of oxygen molecules available for the process. The more oxygen that is present, the more of these pathways, or the more “quenching,” exists. Therefore, the phosphorescent lifetime of the oxygen sensor is inversely proportional to the amount of oxygen present.

Emitted light from the sensor was captured by the same high speed camera on the inverted microscope used for the toxicity experiment. The images were processed with a previously developed in-house program and a lifetime measurement was made [2]. The program output a graph of molecular oxygen parts per million versus time. In order to convert the data to fmol/min, the volume of each well had to be determined; because the wet etch manufacturing process resulted in wells with slightly different volumes, each well had to be measured and its volume corroborated with the data from the cell in that well.

A scanning profilometer was used to determine well volume; however because the stylus could not fit inside the glass microwells, an inverted form of the well was made in PDMS (Sylgard 184, Dow Corning, Midland, MI) so that the profilometer could better detect surface characteristics. Scans were made of the inverse molds at various diameters across the surface. The volume of the well was then calculated and used to determine the oxygen concentration of each individual well with fmol/min resolution.

RESULTS AND DISCUSSION

Cell Trapping

The single-cell self-assembly method was tested by pumping medium containing ~267,000 A549 cells per mL at 3 mL per minute for 6 minutes over a glass chip with 9 arrays of micro-trap groups spaced 2.5 mm apart. The number of traps containing zero, one, and multiple cells was recorded each minute. The measured results for this experiment are reported in Figure 4. The overall self-assembly yield for single-cells was 61% for a total of 81 traps.

At the bottom of each patterned trap there was a gap to allow fluid flow through the structure. If the trap was empty, fluid flowed to the left, right, or down the center and out of the fluid exit port. However, when a cell entered a trap, it blocked the fluid exit port. This limited the fluid flow to sides of the trap. Since the cells were easily manipulated by the fluidic forces, any subsequent cells that came upon an occupied trap were diverted and continued on to the next row. We expected that this design would prevent a single trap from catching multiple cells and result in an array of single-cells.

In practice, however, the longer the self-assembly process continued the number of traps containing multiple cells increased. We hypothesize two reasons for this. First, the cells may not have been completely separated before beginning the flow process. A pair of cells may have been stuck to one another and flowed in to the trap at the same time. The second possibility for multiple cells in a single trap occurred when a smaller cell entered the trap and did not completely block the fluid exit port. This would still allow some fluid to flow through the center of the trap and carry any cells with it.

SU8 Toxicity

The results for the SU8 toxicity experiment are shown in Table 1. A representative fluorescent image of live cells attaching to the glass chip containing patterned traps is shown in Figure 5. For each cell line, cell growth and viability were observed and SU8 was found to be non-toxic to A549 and CP-DhTRT cells.

Oxygen Measurement

A549 cells were used to demonstrate the method of measuring the oxygen consumption rate of single live cells using the SU8 traps. This experiment was repeated multiple times in order to determine the average oxygen consumption rate of A549 cells and to demonstrate repeatability. Figure 6 shows a representative drawdown experiment of three live cells. Each cell was from a separate array measured on separate days and results were compiled onto the same graph for clarity. Results show an oxygen consumption rate of 0.82, 0.83, and 0.71 fmol/minute for single A549 cells, consistent with results using the previous oxygen detection system of 0.91 ± 0.39 fmol/min for A549 cells [3].

CONCLUSION

This method demonstrates a yield of 61% of single-cells captured in SU8 traps using microfluidics. A systematic toxicity experiment was conducted with two cell lines to demonstrate that SU8 does not interfere with normal cell behavior. A representative experiment measuring the oxygen consumption rates of single live cells was performed in order to demonstrate the effectiveness of the system. This method and our validation experiments open the way for building large arrays of traps localizing individual cells in order to conduct various “single-cell” biological experiments.

Acknowledgments

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References

1. Lidstrom ME, Meldrum DR. Life-on-a-chip. *Nature Reviews Microbiology* Nov;2003 1:158–164.
2. Molter TW, Holl MR, Dragavon JM, McQuaide SC, Anderson JB, Young AC, Burgess LW, Lidstrom ME, Meldrum DR. A New Approach for Measuring Single Cell Oxygen Consumption Rates. *IEEE Transactions on Automation Science and Engineering* Jan;2008 5(1)

3. Molter TW, McQuaide SC, Suchorolski MT, Strovas TJ, Burgess LW, Meldrum DR, Lidstrom ME. A microwell array device capable of measuring single-cell oxygen consumption rates. *Sensors and Actuators B: Chemical*. 2008;10.1016/j.snb.2008.10.036

Figure 1.
A) Empty 3x3 array of micro-traps. B) Image of a trapped single-cell. C) Full array of trapped and attached cells. D) Fluorescence image of the array shown in C highlighting the live cells.

Figure 2. Illustration of the single-cell self-assembly set-up. SU8 was patterned on to a glass chip and later sealed with a glass ceiling. Medium containing cells was then flowed through the microchannel to allow the traps to capture individual cells.

Figure 3.

Oxygen measurement: A) Single micro-trap group is sealed using a motorized piston connected to a glass ceiling with corresponding inverted microwells that contain platinum porphyrin oxygen sensor. B) The microwells create a gas impermeable microchamber for each individual cell. After excitation of the phosphorescent oxygen sensor, the emitted light is collected and processed to determine oxygen concentration.

Figure 4. Measured yield results for single-cell self-assembly process using A549 cells after six minutes with a density of ~267,000 cells per ml. 61% of the traps isolated individual cells after 6 minutes

Figure 5.
A representative toxicity image is of A549 cells two days after seeding onto a patterned SU8 chip.

Figure 6. Measured oxygen consumption rates of three trapped single cells. Each data set was taken separately and combined on the same graph for clarity. A calculated oxygen consumption rate of 0.83, 0.71, and 0.82 fmol/minute was measured for sensors 6, 7, and 9, respectively after converting the values of the above graph from ppm to fmol using the individual well volume.

Table 1

SU8 toxicity results (fluorescently labeled, imaged, and counted) with A549 and CP-DhTRT cells two and three days after seeding.

	Live	Dead	Live/Dead	% Dead
A549	Two Days After Seeding			
No Su8	8535	63	135.5	0.73%
Flat SU8	4412	79	55.8	1.76%
Patterned SU8	4001	137	29.2	3.31%
	Three Days After Seeding			
No Su8	9448	44	214.7	0.46%
Flat SU8	13037	222	58.7	1.67%
Patterned SU8	7574	87	87.1	1.14%
CP-DhTRT	Two Days After Seeding			
No Su8	4185	87	48.1	2.04%
Flat SU8	1353	25	54.1	1.81%
Patterned SU8	2884	42	68.7	1.44%
	Three Days After Seeding			
No Su8	8247	46	179.2	0.55%
Flat SU8	2466	49	50.3	1.95%
Patterned SU8	3394	73	46.5	2.11%