

Microfluidic Single-Cell Assay Chip for Drug Efficacy Test

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Abstract:

Single-cell resolution assays incomparably increase the power of drug screens over conventional colorimetric assays by providing quantitative data. Assays of this class avoid cell-to-cell interactions present in traditional bulk sampling procedures; these interactions can mask nuances where individual cells are concerned, invariably causing rich amounts of information to be overlooked. Advances in microfluidic technology have allowed high throughput single-cell drug screens to be performed while exercising precise control over cell loading and culturing conditions with relatively insignificant amounts of expensive samples and reagents used. This project focused on optimizing the design and operation of our drug screening platform for single-cells and neurospheres. Microfluidic chips were fabricated via polydimethylsiloxane (PDMS) replication and bonding. Glioblastoma multiforme (GBM) cells—stained with green fluorescent protein (GFP)—were then introduced to the devices and, upon their capture within individual microwells, cultured to the neurosphere stage while being subjected to drug screens alongside control groups. Significant statistical data of cell viability can be obtained when the starting single-cell number in the chip is greater than 100 and after these cells have been cultured in the chip for at least five days.

Introduction:

Cell-based biological assays, such as drug screens, are notorious for frequently providing the mean data across an entire population of cells, despite the now ubiquitous knowledge that isolated cells, even those belonging to related cell lines, portray extremely diverse properties [1]. Advancements in microfluidic single-cell assay technologies provide biologists with a media through which they can tackle the most adamant challenges.

The quest for finding the cure to cancer is as much a race against time as it is against money, but by utilizing the properties of microfluidic PDMS chips the burden on humanity and on its coffers can be significantly reduced. These inexpensive chips can be used to segregate cells into discrete compartments, allowing many types of quantitative assays to be performed. This project focuses on optimizing the design and operation of a microfluidic single-cell drug screening platform for GBM cells.

Currently, GBM is the most common type of primary brain tumor and only palliative treatments exist. With this design, it is possible to culture single GBM cells to the neurosphere stage, allowing drug screens to account for the aging of cells, something previously unaccomplished. This design further prevents cell-to-cell interactions by providing a constant flow of fresh medium over the cells.

Microfluidic Chip Fabrication and Preparation. Photolithography of SU-8 on silicon wafers was used to create

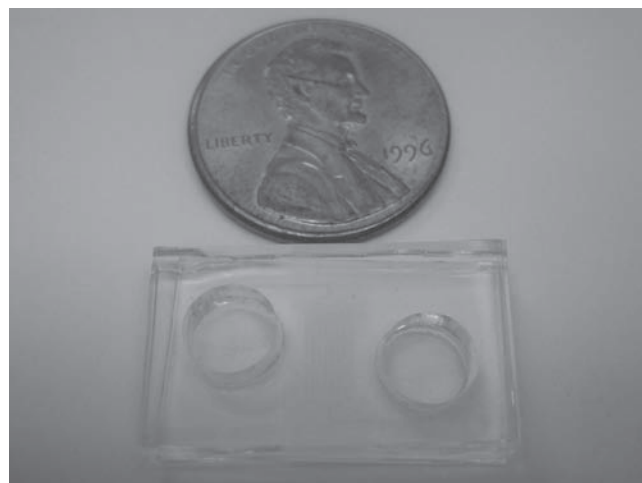
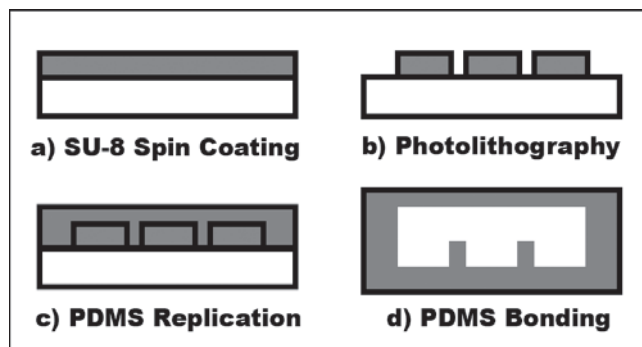


Figure 1, top: Microfluidic PDMS chip fabrication.
 Figure 2, bottom: Microfluidic PDMS chip.

master molds for the channel (top) and microwell (bottom) halves of the PDMS chips (Figure 1). Input and output holes were punched into the channel half of a PDMS chip and then the two halves were treated with oxygen plasma and bonded (Figure 2). Cell-free medium was inserted at the input (80 μ L) and output (40 μ L) wells of the chip which was then degassed in a vacuum chamber to flush air bubbles from the channels.

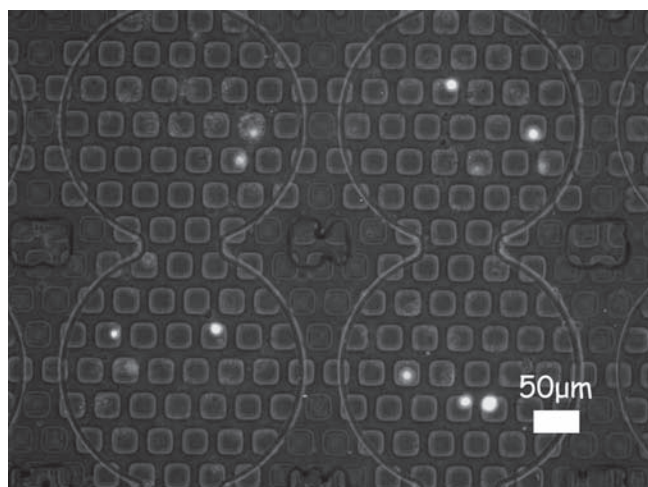


Figure 3: GBM single-cells.

Cell-Loading Procedure. The GBM neurospheres in the cell-containing medium were then manually disassociated to single-cells using a micropipette. The cell-free medium was removed from the input and output wells of the chip; cell-containing medium (80 μ L) was then inserted at the input well. The medium was allowed to flow for 10 minutes before being removed from both wells; the cells in the microfluidic chip were allowed to settle for three minutes. The chip was then inspected with ultraviolet light (10X magnification) for neurospheres stuck in the channels, which were removed by providing negative pressure at the output well via pipette bubble suction. The single-cell captures were then counted manually (Figure 3). If the chip contained less than 100 single-cell captures, more cell-containing medium (80 μ L) was inserted at the input well and the process was repeated until at least 100 cells were captured.

Drug Screening. Once 100 cells were captured, the cells were subjected to drug screens. For the control group, cell-free medium (80 μ L) was injected into the inlets and the cells were cultured for more than five days while being subjected to a continuous flow of fresh medium from inlet to outlet via gravity difference. Chips were kept in an incubator set at 37°C, 5% CO₂ and 90% RH; fresh medium was replaced and cell viability recorded via GFP staining every 12 hours. For the treatment group, cell-free medium containing the drug (GSI) was used instead. Statistics were obtained for cell viability.

Results and Discussion:

The cell-loading procedure was first optimized using microbeads (15 μ m) of similar size to GBM cells (Figure 4). The microbeads were most efficiently captured with the following: channel height, 22 μ m; microwell diameter, 20 μ m, microwell depth, 26 μ m; bead concentration, 2.75×10^6 beads/mL. For the GBM cells, slight dimensional tweaking was required to compensate for the cells' affinity to each other: channel height, 37 μ m; microwell diameter, 30 μ m; microwell depth, 26 μ m. As in the microbead experiments, the cell-loading procedure was most efficient when a high concentration of cells (greater than 10^6 cells/mL) was used, with which capturing over 100 cells became trivial. Preliminary drug screenings were unsuccessful at distinguishing a significant difference between viability in control (34.74%) and treatment group cells (34.84%). More frequent medium changes may be necessary for future screenings.

Acknowledgements:

Infinite gratitude goes to Professor Euisik Yoon and his research group for taking me as an intern and to Xia Lou for the fantastic mentoring that will be forever useful in future endeavors. Great thanks to Dr. Tom Bersano for his assistance with the cell-loading procedure. Much appreciation to the National Nanotechnology Infrastructure Network Research Undergraduate Experience Program, the National Science Foundation, and the wonderful staff at the Lurie Nanofabrication Facility for giving this biologist a taste of engineering.

References:

- [1] Sims, C. E., and N. L. Allbritton. "Analysis of single mammalian cells on-chip". *Lab on a Chip*, 7, 423-440 (2007).

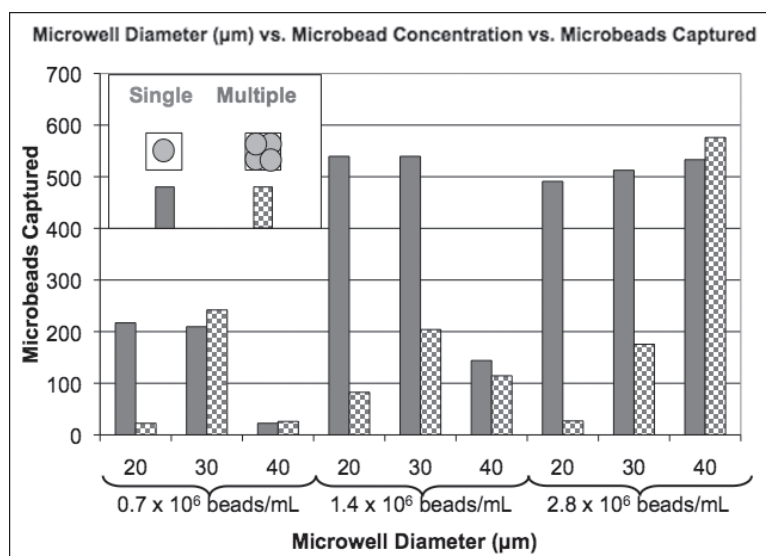


Figure 4: Optimization of microbead capture.