Novel Microfluidic Chip for Reproducible Formation of Organoids

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Problem

Organoids are three-dimensional miniature organs derived from stem cells that self-form *in vitro* when cultured in an appropriate environment (Figure 1) [1-3]. Because organoids more accurately mimic physiological processes compared to 2D-cultured cells, they present a promising opportunity for drug testing and disease modelling. However, current methods of organoid formation are inconsistent, time-consuming, and not reproducible. More specifically, brain organoid formation is a fragile procedure since it requires the use of neural progenitor cells (NPCs), which are extremely delicate cells that can differentiate into certain neural and glial subtypes [4]. Thus, this project aimed to develop a microfluidic device that can evenly distribute human-induced pluripotent stem cell (hiPSC)-derived NPCs to microwells, producing reliably formed and identical organoids.

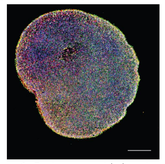

Figure 1: Brain organoid showing glial cells (magenta), astrocytes (green and red), and cell nuclei (blue). Scale bar 425µm. [3]

Project Goals

Constraints

1. The system must contain at least one inlet and one outlet for media exchange.
2. The system must be compatible with a syringe pump.
3. The prototype must be fabricated from polydimethylsiloxane (PDMS).

Objectives

1. The system is sealed and does not leak.
2. The system evenly distributes media to the microwells within 5% deviation.
3. 80% of injected cells are retained in the device.

Methodology

1. To eliminate leakage, a variety of design and manufacturing modifications were investigated, including:
   a. Increasing channel dimensions to decrease internal pressure during loading.
   b. Implementing a resin casing surrounding the PDMS layers to improve pressure distribution.
   c. Applying silicone-based lubricant as a flow deterrent on PDMS.
   d. Placing a glass slide on curing PDMS to ensure it is flat without artifacts.
   e. Adjusting mold geometry to ensure PDMS layers cure flat and without artifacts.
2. To confirm resolution of leakage and feasibility of future testing with cells, a syringe pump was used to inject coloured water into the device at flow rates from 5µL/min to 2000µL/min [5] for a channel width of 2.0mm. Fluid distribution and number of microwells filled were compared at each flow rate.
3. To test for cell retention in the device and cell viability, five flow rates in the range of 50µL/min to 2000µL/min were tested with hiPSC-derived NPC-laden cell culture media in two channel widths (2.0mm and 2.5mm). These results were contrasted to find the optimal loading volumetric flow rate and PDMS component combination. Cell concentrations and viabilities were determined before and after injection using an automated cell counter and trypan blue staining.

Final Design

The final design consists of a PDMS microfluidic device composed of channels that direct fluid to identical microwells*. These microwells are intended to house and reproducibly form prospective organoids. The entire device is enclosed in a 3D-printed resin casing with inlets and outlets for cell culture media (Figure 2). The casing is tightened using eight peripheral screws to maintain pressure and ensure contact between the PDMS layers, preventing leakage. The final prototype was created by designing 3D-printed resin molds and the resin casing using SolidWorks software. The microfluidic chip was then fabricated by pouring PDMS into the molds. Two different channel widths of 2.00mm and 2.50mm were designed and tested to determine the effect of channel geometry on cell retention and viability (Figure 3).

**Images of the full prototype have been excluded for confidentiality purposes at the request of Team Progenitor from the Willerth Lab.*

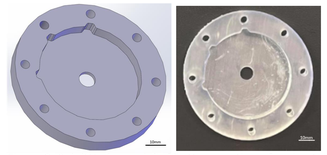

Figure 2: SolidWorks model of bottom portion of casing to enclose microfluidic device (left) and 3D-printed resin prototype (right). Holes along circumference are used for screw insertion while center hole is intended for cell culture media injection.

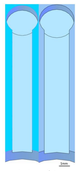

Figure 3: SolidWorks models of two microfluidic channel designs: 2.0mm channel width and 3.2mm microwell diameter (left), 2.5mm channel width and 3.5mm microwell diameter (right). The channels direct media towards the microwells, which house organoids.

Results

Cell retention and cell viability were assessed for flow rates varying from 50 to 2000 µL/min and for two different channel dimensions. It was found that an increase in flow rate resulted in a decrease in cell retention but did not affect cell viability. After running a two-tailed Student's t-test (95% confidence interval), the following conclusions were made:

- No significant difference between the two channel sizes with respect to cell retention was found.
- However, the thicker channels maintained significantly higher cell viability compared to the thinner channels at the 100µL/min flow rate.
- There were significant differences between the two channels with respect to the number of channels that fully filled with the injected fluid. These significant differences were found at flow rates of 100µL/min and 1000µL/min, for which the thinner channels outperformed the thicker channels.

The optimal flow rate was 50µL/min, which yielded cell retention of 68.4 ± 1.7% in the 2.0mm channels and 73.0 ± 20.1% in the 2.5mm channels.

To determine if there was a significant difference between flow rates within each channel dimension, an ANOVA test with Tukey post-hoc analysis (95% confidence interval) was performed. From these tests, the following conclusions were made:

- For the number of channels filled, no significant difference was found at different flow rates for either channel dimensions.
- For viability with the 2.0mm channels, there was a significant difference between the following flow rates: 50µL/min vs. 100µL/min and 100µL/min vs. 1000µL/min.
- No significant difference in viability was found at the various flow rates with the 2.5mm channels.
- For cell retention, a significant difference was found at the following flow rates (µL/min) for the 2mm channels: 50 vs. 500 and 50 vs. 1000; and for the 2.5mm channels: 50 vs. 500, 50 vs. 1000, 50 vs. 2000, and 100 vs. 2000.

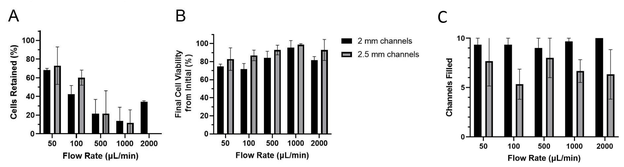

Figure 4. Cell suspension was injected into the device for two different channel sizes using a syringe pump at varying flow rates. Cell concentration and viability were assessed using a cell counter and trypan blue staining. (A) Cell concentration of ejected media was compared to the initial concentration to calculate cells retained. (B) Cell viability of expelled media was compared to initial viability of injected cells. (C) Number of channels filled at each flow rate was examined.

Conclusions

Two PDMS microfluidic device prototypes designed for reproducible organoid formation were tested that had different channel widths (2.0mm and 2.5mm) and met all necessary constraints. For both prototypes, the system was sealed and did not leak; thus, the first objective of the project was met.

Furthermore, cell-based tests were executed by injecting hiPSC-derived NPCs into the device and determining cell retention and viability at various flow rates. It was noted that cell retention decreased with increasing flow rates, which ranged between 50µL/min and 2000µL/min. Cell viability remained at or above 80% for most of the flow rates tested.

Although none of the flow rates tested achieved the target of 80% cell retention, the most optimal flow rate was determined to be 50µL/min. This flow rate attained 68.4 $\pm$ 1.7% cell retention with the 2.0mm channels and 73.0 $\pm$ 20.1% retention with the 2.5mm channels. Additionally, in comparison to thicker channel dimensions, it was noted that the thinner channels more reliably filled a greater number of microwells. This increased reliability is imperative for attaining the objective of even media distribution.

It is recommended that future investigations analyze the effect of decreasing the channel widths and flow rates while implementing improvements to the usability, sterilizability, and imaging potential of the device. In addition, verification of even cellular distribution and organoid formation will require prioritization in the future in order to achieve all of the design aims.

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