Microfluidic single-cell encapsulation
Towards a spatially controlled three-dimensional neuronal culture for brain on a chip technology

Brain on a chip technology, for obtaining more knowledge about the physiology of both the healthy and diseased human brain, is currently being developed at Eindhoven University of Technology [1]. One of the main challenges is to engineer a system that promotes the formation of a physiologically relevant neuronal network in 3D, while retaining controllability, observability, and reproducibility. Here, a novel approach to control the 3D spatial distribution of neuronal cells is presented, based on the encapsulation of single SH-SY5Y cells in Matrigel beads and subsequent bead self-assembly. To tackle this challenge, a microfluidic device for Matrigel bead generation was designed, after which and single-cell encapsulation, bead self-assembly, and cell behavior in an assembled culture were investigated.

Microfluidic generation of Matrigel beads

A microfluidic flow-focusing chip with integrated temperature control chambers was designed for generating Matrigel droplets below 4°C, and subsequent droplet gelation at 37°C to form beads.

Highly monodisperse Matrigel droplets with dispersity <3% and a radius of approximately 48.4 µm could be reproducibly generated using the microfluidic device. After gelation, the beads had a slightly higher dispersity of 4.4%.

Microfluidic encapsipation of cells in Matrigel beads

Cell were encapsulated by adding them to Matrigel before bead generation. Due to the Poisson statistics that govern single-target encapsulation, only 10.6% of the generated beads contained a single cell, 4.1% contained two or more cells, and 85.3% was empty.

Single-cell (top) and double-cell (bottom) encapsulation was observed in the flow-focusing section using a high-speed camera. Cells are indicated with arrows.

For future work, incorporation of a microfluidic method, such as hydrodynamic cell ordering in high aspect-ratio channel flow [2], to overcome the Poisson statistics is recommended.

Self-assembly of Matrigel beads

In mineral oil, the Matrigel beads assembled into a highly organized packing, due to a combination of repulsive bead interactions, and a driving force of approximately 0.87 nN.

When transferred to cell culture medium, less organized packing was observed. This effect is likely caused by attractive interactions between beads and a lower driving force for sedimentation, which is estimated at 0.05 nN.

Some beads had aggregated into clusters

Locally, hexagonal packing was observed

For future work, a microfluidic assembly method or a change of encapsulation material is recommended to achieve better organization of Matrigel beads.

Spatially organized neuronal culture

Most of the SH-SY5Y cells cultured in Matrigel beads remained viable until at least day 9, as indicated by viability live-staining, where Nucblue stains live cells (blue) and Nucgreen stains dead cells (green).

A single encapsulated SH-SY5Y cell is shown, with a neurite of over 100 µm extending out of the bead (left). Differentiation was further shown by antibody staining for β-tubulin III (red), a neuron specific cytoskeletal protein (right). Here, the cell nuclei are stained with DAPI (blue). Neurites are indicated with white arrows.

Conclusions

In this work, SH-SY5Y cells have been successfully encapsulated in Matrigel microbeads, without significantly affecting their viability and differentiation potential. When the proposed improvements are implemented, the level of Matrigel bead organization can be greatly improved, such that full control over the spatial organization of differentiated neurons can be achieved.