**A 3D-printed model of the subarachnoid space to study the role of meningothelial cells during optic nerve compartmentalization**

**Subashree Srinivasan**, Corina Kohler, Claudia Bippes, Hanspeter E. Killer, Albert Neutzner

Department of Biomedicine, University Hospital Basel and University of Basel, Basel, Switzerland

Abstract—The cerebrospinal fluid (CSF)-filled subarachnoid space (SAS) between the arachnoid and pia mater is lined by meningothelial cells (MECs) and are in direct contact with flowing CSF. MECs are thought to contribute to maintaining CSF equilibrium by secreting proteins and eliminating neurotoxic waste via endocytosis. As CSF homeostasis is critical for neuronal function, MECs are deemed neuroprotective. Therefore to study MEC metabolism during optic nerve compartment syndrome, we developed a 3D-printed, perfusable SAS model compatible with confocal microscopy under (patho)physiological flow conditions.

Keywords— Meningothelial cells, Cerebrospinal fluid, Subarachnoid space, Optic nerve compartment syndrome, bioreactor model.

INTRODUCTION

Like the brain, the optic nerve is covered and protected by three meningeal layers. The cerebrospinal fluid (CSF)-filled subarachnoid space (SAS) between the arachnoid and pia mater is lined by meningothelial cells (MECs) and are in direct contact with flowing CSF. MECs are thought to contribute to maintaining CSF equilibrium by secreting proteins and eliminating neurotoxic waste via endocytosis [1]. As CSF homeostasis is critical for neuronal function, MECs are deemed neuroprotective. During optic nerve compartment syndrome (ONCS), the SAS becomes separated from the primary CSF volume, significantly restricting CSF flow leading to visual impairment associated with normal-tension glaucoma, papilledema, and intracranial pressure syndrome in astronauts [2]. Our previous research revealed that altered perfusion conditions impact MEC metabolic activity [3]. To overcome the limitations of commercial bioreactors, we developed a 3D-printed, perfusable SAS model to mimic ONCS enabling us to study MEC metabolism using confocal microscopy and fluorescent-based biosensors under (patho)physiological flow conditions.

Materials and Methos

Leveraging 3D bioprinting, gelatin methacrylate (GelMA) microchannels with ~100µm pillar-like structures resembling those in the SAS were successfully created. A self-contained microscope-compatible perfusion setup, including a GelMA microchannel, media chamber, bubble trap, pressure sensor, and piezoelectric micro-pump, was developed. A user-friendly interface was developed to allow precise perfusion control and monitoring to culture immortalized and primary MECs expressing fluorescent biosensors on GelMA microchannels.

Results

We find that GelMA microchannels demonstrate biocompatibility, forming and maintaining tight MEC monolayers for one month in culture. Our perfusion model ensures a high level of cell viability within a sterile, leak-free system. The established pressure-flow relationship in the model highlights the reproducibility of the complete perfusion system. Utilizing live-cell microscopy and a genetically encoded biosensor, we measured the impact of normal and pathological flow conditions on ATP levels in MECs.

CONCLUSIONS

Currently, we evaluate flow effects on MEC metabolic factors such as mitochondrial membrane potential, ATP production, and NADH-NAD+ ratios. Our novel bioreactor enables live-cell imaging, facilitates the investigation of MEC response and adaptations, potentially uncovering ONCS and optic nerve disease mechanisms. Additionally, our 3D perfusion system extends utility beyond the SAS, offering a platform for general flow-related cell behavior research.

REFERENCES

[1] J. Li, L. Fang, H. E. Killer, J. Flammer, P. Meyer, and A. Neutzner, “Meningothelial cells as part of the central nervous system host defence,” *Biol. Cell*, vol. 105, no. 7, pp. 304–315, Jul. 2013, doi: 10.1111/boc.201300013.

[2] H. E. Killer, “Compartment syndromes of the optic nerve and open-angle glaucoma,” in *Journal of Glaucoma*, Jun. 2013, vol. 22, no. 5 SUPPL.1, doi: 10.1097/IJG.0b013e3182934a0f.

[3] A. Neutzner *et al.*, “A perfusion bioreactor-based 3D model of the subarachnoid space based on a meningeal tissue construct,” *Fluids Barriers CNS*, vol. 16, no. 1, Jun. 2019, doi: 10.1186/s12987-019-0137-6.