Engineering an organ-on-chip device to study brain organotropism in lung cancer metastasis

Tamagno Pesqueira[1,2,3], Sara Abalde-Cela,[1,4] Elena Martínez [2,3], Lorena Diéguez [1,4]
[1]International Iberian Nanotechnology Laboratory, Braga, Portugal
[2]Institute for Bioengineering of Catalonia, Barcelona, Spain
[3]University of Barcelona, Barcelona, Spain
[4]RUBYnanomed Lda., Braga, Portugal

jose.tamagno@inl.int

Cancer is characterized by abnormal cell proliferation and growth, leading to the invasion of other tissues and organs via the bloodstream and lymphatic systems, a process central to the hallmarks of cancer [1]. Metastasis - the dissemination of cancer cells to distant locations - represents the most devastating stage of the disease and is responsible for the majority of cancer-related deaths [2]. Lung cancer, particularly non-small cell lung cancer (NSCLC), is the leading cause of cancer-related deaths worldwide, with an estimated 1.8 million deaths annually. The prognosis for metastatic NSCLC remains poor, with a 5-year survival rate of only 5% and a median progression-free survival of 8-14 months [3,4]. Notably, 25-30% of NSCLC patients present with advanced or metastatic disease at the time of initial diagnosis. Autopsy studies reveal distinct patterns of metastatic spread, with secondary sites most commonly found in the brain (39 %), followed by bone (34 %) and liver (21 %) [5]. Indeed, this metastatic spread is not random but exhibits organotropism, where disseminated cancer cells preferentially colonize specific secondary organs depending on the primary tumor of origin. Therefore, understanding the mechanisms driving organotropism remains an unmet clinical question in cancer research, and is essential for improving outcomes for patients with advanced NSCLC.

While in vivo models, such as mice and humanized mice, offer insights into the complex mechanisms of metastasis, they often fail to acutely replicate human disease pathophysiology, leading to inconsistent outcomes. In vitro models, particularly those utilizing microfluidics, have emerged as powerful tools to study cancer biology by precisely emulating physiological environments in a controlled manner [6]. These models have enabled researchers to unveil key mechanisms involved in the extravasation of circulating tumor cells, both at cellular and molecular levels [7, 8, 9].

Therefore, the objective of this work is to engineer an organ-on-chip device of the brain tropism to study the extravasation and invasiveness of NSCLC cells with distinct phenotypes. The proposed microfluidic device aims to emulate the physiological brain microenvironment, specifically the physical and biological characteristics of the blood-brain barrier (BBB). The integrated microfluidic device incorporates key engineering design considerations and biological features (Figure 1) to investigate how physiologically relevant hemodynamic forces influence the extravasation of NSCLC cells across the BBB.

The microfluidic device was designed using computer-aided design software and fabricated through soft-lithography. It consists of a single channel with rectangular geometry with high width-to-height aspect ratio enabling laminar flow and negligible wall effects, as confirmed by computational fluid dynamics (CFD) simulations. The optimized design was 3D-printed using stereolithography, then used as a mold for polydimethylsiloxane-based soft-lithography, and plasma bonded to glass coverslips.
Human brain microvascular endothelial cells (HBMECs) were characterized under static conditions to determine growth parameters and seeded into the microfluidic devices at optimized density. Immunocytochemistry confirmed formation of a confluent monolayer 72-hour post-seeding as observed by the expression of tight-junction protein (ZO-1). CFD analysis was used to simulate and calculate the shear stress experienced by the HBMECs inside the microfluidic device compared to the theoretical values of an empty channel. The microfluidic device was connected to a flow system to expose the HBMECs to physiological shear stress of 0.6 Pa for 48 hours, replicating in vivo BBB conditions.

In summary, we have successfully engineered a microfluidic device featuring a functional monolayer of HBMECs that replicates basic biomechanics of the BBB based on the optimal dynamics for cell growth. This device provides a foundation for future studies investigating the extravasation potential of NSCLC cells with distinct phenotypes (i.e., metastatic abilities), as well as quantitative analysis of metabolite release and cellular nanomechanics.

References

[1] Hanahan, D., Cancer Discovery, 12 (2022) 31-46
[2] Nguyen, D. *et al.*, Nature Reviews Cancer, 9 (2009) 274-284
[3] Belaroussi, Yaniss *et al.,* Sci. Reports, 13 (2023) 9584
[4] Hirsch, Fred R. *et al.,* The Lancet, 389 (2017) 299-311
[5] Gao, Y. *et al.*, Development Cell, 49 (2019) 375-391
[6] Sontheimer-Phelps, A. *et al.,* Nature Reviews Cancer, 19 (2019) 65-81
[7] Kim. H. *et al.,* Advanced Science, 6 (2019) 1900195
[8] Kim, J. *et al.,* ACS Nano, 14 (2020) 14971-14988
[9] Hajal, C. *et al.,* Biomaterials, 265 (2021) 120470

Figures

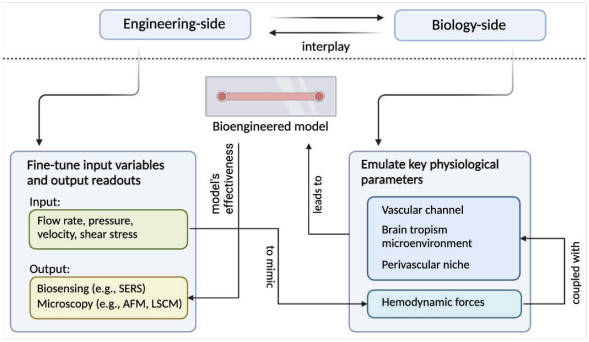

Figure 1. Workflow of the bioengineered blood-brain barrier model for studying the extravasation of lung cancer cells, integrating engineering considerations and biological features of the physiological tissue.