Lymphangiogenesis on a chip: quantifying sprouting responses to inflammatory stimuli

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Summary

Lymphangiogenesis on a chip:
Quantifying sprouting responses to inflammatory stimuli

Master's thesis submitted by

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Summary

The lymphatic system is indispensable for tissue fluid homeostasis, immune surveillance, and lipid absorption. These functions rely on the proper functioning of lymphatic vessels, including their valves and drainage mechanisms, and are also linked to lymphatic remodeling driven by the dynamic activity of lymphatic endothelial cells (LECs). The LECs migrate, sprout, and form new vessels through the process of lymphangiogenesis. This process is essential during development and tissue repair but is also critically involved in pathological contexts such as chronic inflammation, cancer metastasis, and lymphedema. Understanding how lymphangiogenesis is regulated under both physiological and pathological conditions is therefore fundamental to biological research and the development of therapeutic strategies.

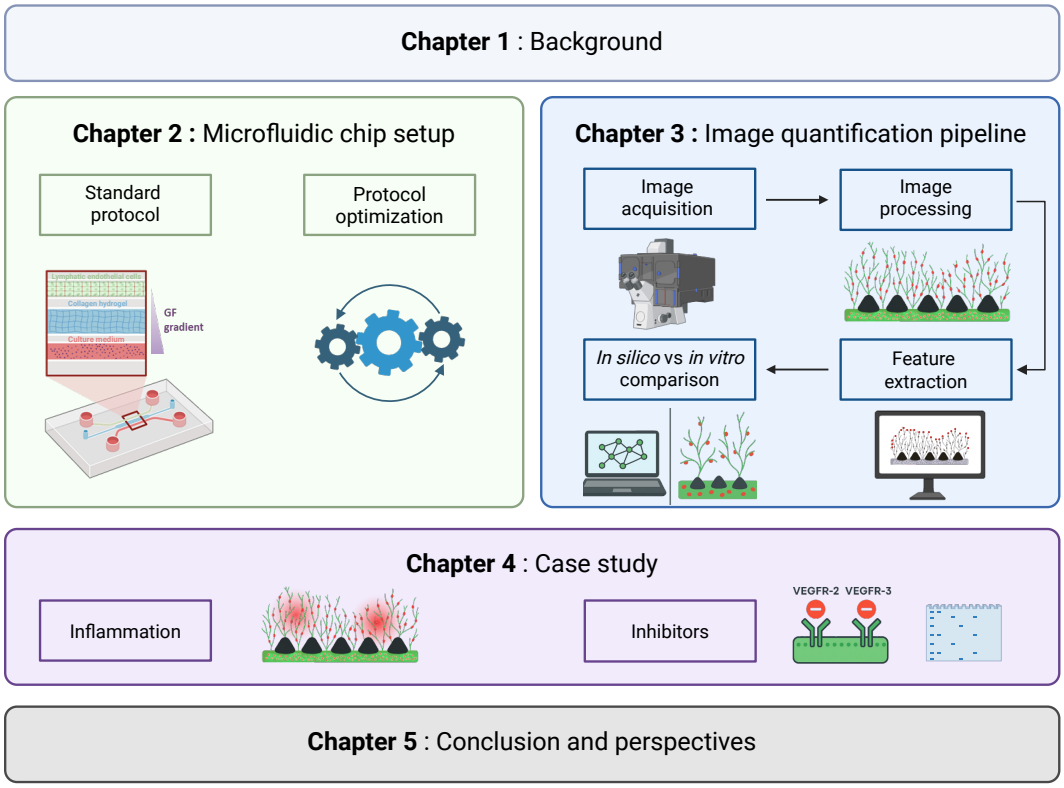
Despite the biological importance of lymphangiogenesis, the experimental study of this process remains challenging. Conventional 2D *in vitro* assays oversimplify the microenvironment and fail to reproduce its structural and biochemical complexity. *In vivo* models, although physiologically relevant, are constrained by ethical considerations, high costs, and limited experimental control. Advanced 3D *in vitro* systems offer greater physiological relevance but often remain difficult to standardize and interpret. In this context, microfluidic organ-on-chip platforms have emerged as promising tools. They enable application of biochemical gradients, integration of extracellular matrix, and incorporation of flow, thereby providing a more reproducible and physiologically relevant setting. However, the challenge does not only lie in developing suitable models but also in quantitatively analyzing the data they generate. Imaging plays a central role in these systems by providing detailed visual information on lymphatic sprouting and network organization. Nevertheless, despite significant advances in imaging technologies, robust methods to convert complex image datasets into quantitative descriptors remain limited. Existing image analysis tools, largely adapted from blood vasculature, are not optimized for lymphatic morphology and often restrict interpretation to a qualitative level. This emphasizes the need for analytical approaches designed specifically for lymphangiogenesis and customized to the constraints of our platform.

This master's thesis addresses these challenges through a dual approach. First, a microfluidic lymphangiogenesis-on-chip platform was established based on a standard protocol. This protocol was then optimized to ensure reproducibility and robust induction of LEC sprouting. Protocol refinements, including optimized seeding and incubation strategies, resulted in more consistent and relevant sprouting responses. Second, an image quantification pipeline was developed, comprising image acquisition, processing, and feature extraction steps. This workflow enabled the extraction of morphometric descriptors such as sprout length, actin density, and nuclear distribution from confocal images. Importantly, the pipeline was designed to process not only experimental images but also outputs of a numerical model, thereby creating a quantitative bridge enabling direct comparison between experimental (*in vitro*) and computational results (*in silico*).

To evaluate the applicability and biological relevance of the integrated framework, it was applied to two case studies. In the first case, exposure to pro-inflammatory cytokines (TNF-α and IL-1β) significantly reduced VEGF-driven sprouting, confirming the inhibitory effect of this inflammatory signaling on LEC invasion. In the second case, pharmacological inhibition of VEGFR pathways revealed distinct roles for VEGFR-2 and VEGFR-3. VEGFR-2 inhibition by ZM323881 produced a clear and dose-dependent suppression of sprout formation, whereas VEGFR-3 inhibition by MAZ51 led to more moderate and variable effects. Overall, these findings are consistent with previous reports describing the impact of inflammatory signaling and VEGFR pathways on lymphangiogenesis. They demonstrate the ability of the system to capture complex and biological responses underlying lymphatic sprouting. It should be emphasized, however, that the case studies of inflammation and receptor inhibition primarily served to validate the image analysis pipeline itself rather than to provide an exhaustive biological interpretation of the observed effects.

The guiding objective of this thesis was therefore structured around two complementary aspects: an experimental platform enabling the observation of LEC behavior under control and perturbed conditions, and a computational pipeline capable of translating these observations into quantitative descriptors. This combination provides the basis for iterative refinement of computational models, which could ultimately serve as predictive tools to test perturbations and explore cellular behavior numerically. In this framework, experimental assays would be reserved for the validation of the most promising predictions. Altogether, it advances the methodological framework available for lymphatic research. Beyond immediate results, this framework provides a foundation for predictive and mechanistic insights into lymphatic sprouting. With further refinements, this approach holds potential for the generation, testing, and validation of new hypotheses in lymphatic biology.

Figure 1 provides an overview of the master's thesis structure and its contents.



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