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Modeling vascular inflammation with immune cell-vessel crosstalk in hiPSC-derived 3D vessels-on-chip

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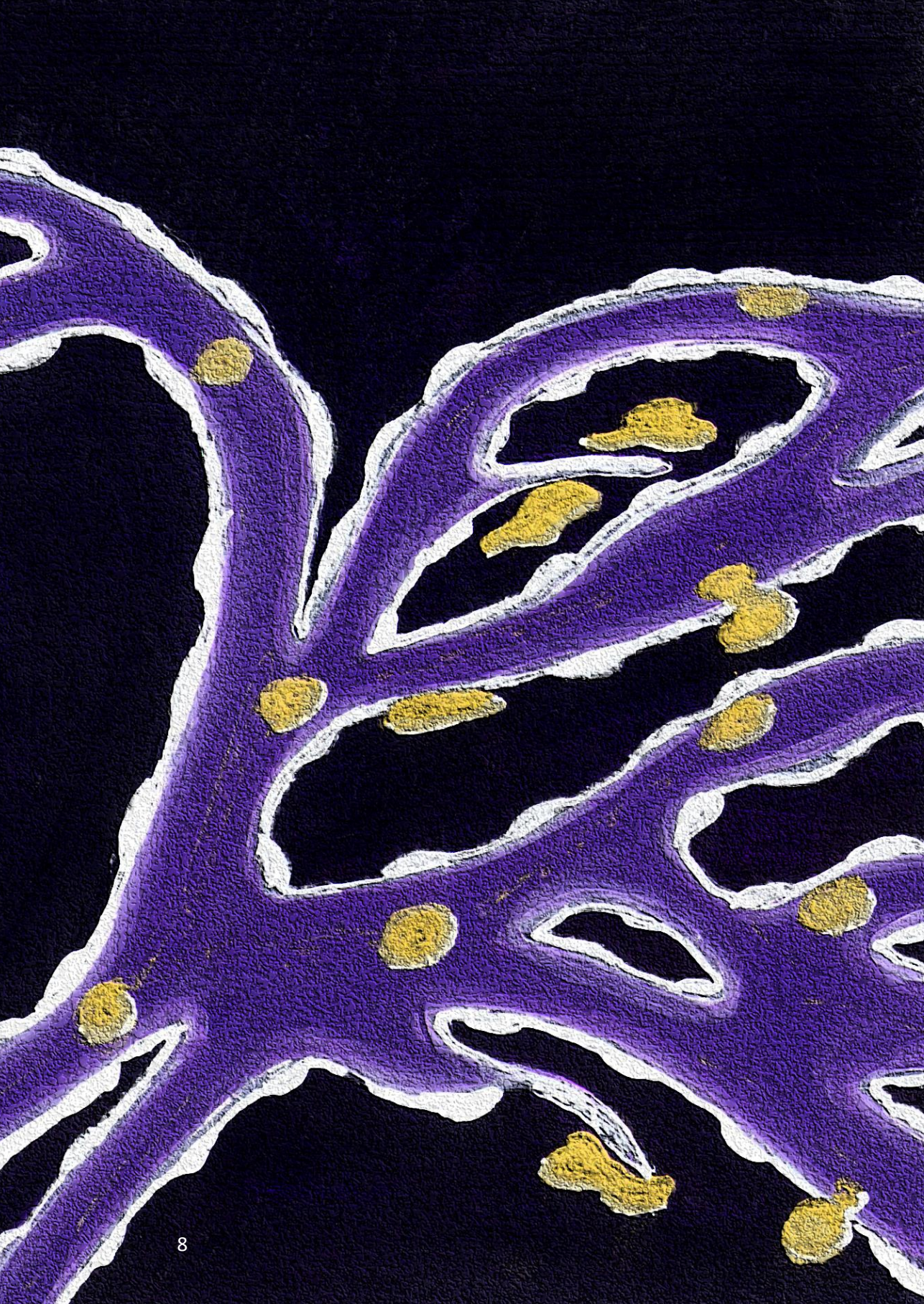

Chapter 1

General Introduction

Introduction

The vascular system plays a key role in organism development, tissue homeostasis, and host defense. Endothelial cells (ECs) and mural cells, including pericytes and vascular smooth muscle cells (vSMCs), collaborate to maintain vascular integrity, adopt tissue-specific functions, and regulate hemodynamics. Inflammatory responses within the vasculature, mediated by ECs and mural cells, are critical for leukocyte recruitment and immune regulation but can lead to vascular dysfunction and disease when dysregulated. Hereditary hemorrhagic telangiectasia type 1 (HHT1), a rare vascular disorder exemplifies the interplay of genetic, cellular and inflammatory factors in vascular pathologies, highlighting the need for advanced models to capture these dynamics.

Recent innovations in vascular disease modeling leverage human induced pluripotent stem cells (hiPSCs) and organ-on-chip (OoC) technologies to recapitulate the vascular microenvironment, including fluidic flow. This enables studies of barrier function, leukocyte transmigration, and disease mechanisms. Research described in this thesis aimed to improve existing vessel-on-chip (VoC) models further by enhancing their complexity and physiological relevance by incorporating hiPSC-derived ECs, mural cells, monocytes, and macrophages. By including hiPSC-derived macrophages, this work also allowed exploration of the roles of these cells as perivascular immune regulators, specifically their contributions to vascular function in both health and inflammation. These advanced models were used to investigate vascular inflammation, leukocyte recruitment, and microvascular development in health and disease contexts.

1. Overview of the Vascular System

1.1 Development of Blood Vessels

The cardiovascular system is the first “organ” to develop in vertebrate embryos, serving a vital role during gestation by delivering oxygen and nutrients to growing tissues while removing metabolic waste. The vascular system originates through vasculogenesis, a process in which ECs, derived from mesodermal angioblasts, coalesce to form the primitive vascular plexus. This network undergoes angiogenesis, a process whereby ECs proliferate, migrate, and sprout from existing vessels to form new ones. These processes are accompanied by the recruitment of mural cells, such as pericytes and vSMCs, which stabilize the vessel walls and regulate vascular function[1,2].

During vascular remodeling, ECs differentiate into distinct subtypes, including arterial, venous, and hemogenic ECs, to establish a hierarchical vascular network comprising arteries, veins, and capillaries (Figure 1). These events are governed by tightly regulated molecular and transcriptional programs. Key transcription factors, such as those in the ETS and Fox families, direct early EC specification and lineage commitment. Specifically, the ETS domain transcription factor Etv2 is a central regulator of endothelial and hematopoietic lineage specification[3–5]. Vessel development and remodeling are further controlled by signaling pathways, including vascular endothelial growth factor (VEGF), Notch, and transforming growth factor- β (TGF-β) pathways. VEGF gradients drive sprouting angiogenesis by guiding EC migration, while Notch signaling balances tip and stalk cell behavior to ensure proper branching and vessel stabilization. The Wnt and TGF-β pathways further contribute to vessel maturation and differentiation. Additionally, environmental factors, such as hypoxia and nutrient availability, influence vascular development and EC specialization[6].

Dynamic and reciprocal interactions with tissue-specific microenvironments drive ECs to adopt specialized functions that maintain vascular integrity, promote organogenesis, regulate tissue homeostasis and regeneration through angiocrine factors[7,8]. For instance, in the brain, ECs form a highly selective blood-brain barrier through interactions with pericytes and astrocytes, while in the liver, sinusoidal ECs adopt a fenestrated structure to facilitate metabolic exchange. These adaptations are mediated by tissue-derived growth factors, extracellular matrix proteins, and mechanical forces, such as shear stress[6].

1.2 Hierarchical Organization and Hemodynamics of the Vascular System

The vascular system is comprised of arteries, veins, and capillaries, each with distinct structural and functional characteristics[9] (Figure 1). Arteries are large-diameter vessels (approximately 4–25 mm) that transport oxygen-rich blood from the heart to peripheral tissues[10,11]. Their walls are thick and elastic, composed of ECs, vSMCs, and extracellular

matrix components, enabling them to withstand and regulate high-pressure and pulsatile blood flow. Wall shear stress in large arteries typically ranges from 10 to 70 dynes/cm² (1 to 7 Pa)[10].

Veins are larger in diameter (approximately 5–30 mm) and have thinner walls compared to arteries. They return deoxygenated blood to the heart under lower pressure, with wall shear stress values between 1 and 10 dynes/cm². The venous wall structure includes ECs, a thinner vSMCs layer, and a more prominent adventitia, facilitating compliance functions[12].

Capillaries are the smallest blood vessels, with diameters ranging from 5 to 10 µm, facilitating the exchange of gases, nutrients, and waste products between blood and tissues. Their walls consist of a single layer of ECs with pericytes, allowing efficient diffusion. Wall shear stress in capillaries typically ranges from 1 to 15 dynes/cm² [13].

1.3. Function of Mural Cells

Mural cells, pericytes and vSMCs, are essential for the development, stabilization, and maintenance of the blood vessels. During vascular development, ECs secrete platelet-derived growth factor-B (PDGF-B), which binds to PDGFR-β on mural cells, driving their recruitment. This pathway is crucial for the association of pericytes with capillaries and venules, as well as the recruitment of vSMC to arteries and veins, facilitating vascular maturation and stabilization[14,15]. Additionally, angiopoietin-1 (Ang1) signaling through the Tie2 receptor and TGF-β further support mural cell attachment, differentiation, and the long-term stabilization of nascent vessels[16,17].

Although pericytes and vSMCs share roles in supporting vascular integrity, they differ in their localization, structure, and specific functions. Pericytes are embedded within the basement membrane of capillaries and venules, forming close physical contacts with ECs. These interactions enable pericytes to regulate endothelial barrier integrity and vascular permeability, particularly in specialized vascular beds like the blood-brain barrier (BBB). Pericytes also influence endothelial quiescence, vascular remodeling, and extracellular matrix deposition, which are crucial for angiogenesis and capillary stabilization[18,19]. vSMCs surround larger vessels, such as arteries and veins, forming organized concentric layers around ECs (Figure 1). Their primary role is to provide contractile force to regulate blood flow and stabilize vessels against high blood pressure[14]. The recruitment and function of both pericytes and vSMCs are critical for vascular function, as disruptions in their behavior can lead to vascular abnormalities, including hemorrhages and aneurysms[14,18].

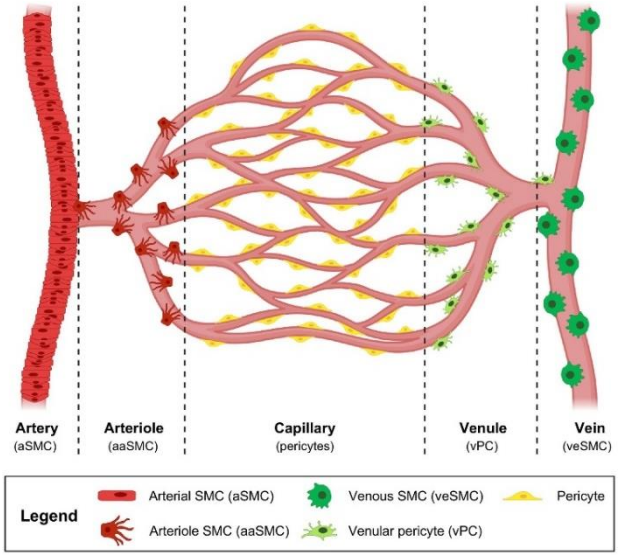

Figure 1. *Schematic representation of the vasculature and the associated mural cells. This figure is adapted from van Splunder et al. (2024)* [20].

2. Inflammatory Responses of the Vasculature

Inflammatory responses are a highly regulated process critical for host defense and tissue repair. While restoring homeostasis following injury or infection, chronic and sustained inflammation can become maladaptive, leading to progressive tissue injury and disease[21]. Blood vessels play a pivotal role in responding to inflammation by facilitating leukocyte transmigration to affected tissues. ECs are central to these processes, transitioning from a quiescent- to an activated state in response to pro-inflammatory stimuli. Through pattern recognition receptors (PRRs), ECs detect danger-associated molecular patterns (DAMPs) and initiate inflammatory signaling cascades, including that of NF-κB, driving the production of cytokines, chemokines, and adhesion molecules. However, excessive or prolonged activation can lead to endothelial dysfunction, exacerbating vascular damage and immune dysregulation[22].

Mural cells also play a critical role in the vascular inflammatory response by contributing to both innate and adaptive immunity. These cells express functional PRRs, enabling them to sense pathogens and tissue damage. They actively shape the local immune microenvironment by secreting cytokines, chemokines, and regulating leukocyte trafficking[23–26].

2.1. Leukocyte Transendothelial Migration (TEM)

Leukocyte trans-endothelial migration (TEM) is a tightly regulated process that enables immune cells to exit the circulation and reach sites of tissue injury or infection (Figure 2). This process predominantly occurs at post-capillary venules. Under physiological conditions, the endothelial lining is anti-adhesive. However, local tissue injury or infection activates ECs, resulting in the upregulation of pro-adhesive receptors on their surface to capture circulating leukocytes.

The process begins with leukocyte tethering and rolling along activated ECs, mediated by selectins (e.g., P-selectin and E-selectin) and their glycoprotein ligands on leukocytes. This transient interaction slows leukocytes, enabling them to engage with pro-inflammatory chemokines presented on the endothelial surface. Firm adhesion is facilitated by leukocyte integrins such as VLA-4 ($\alpha_4\beta_1$), LFA-1 ($\alpha_L\beta_2$), which bind to adhesion molecules expressed on ECs, including VCAM-1 and ICAM-1[27–29]. Integrin activation play a crucial role in both leukocyte adhesion to the endothelium and their subsequent extravasation across vascular beds. Typically inactive under normal conditions, leukocyte integrins are activated via conformational changes triggered by interactions with endothelial-displayed chemokines and the shear stress of blood flow[30,31].

Following firm adhesion, leukocytes migrate laterally along the endothelium, a process known as “crawling”, to locate permissive sites for transmigration. TEM occurs via two main pathways: paracellular migration, when leukocytes pass between adjacent ECs, and transcellular migration, when leukocytes pass directly through ECs. Paracellular migration involves the temporary disruption of intercellular junctions, regulated by adhesion molecules such as PECAM-1, JAMs, and CD99, which coordinate the controlled opening and resealing of junctions to preserve vascular integrity. In contrast, transcellular migration requires the formation of endothelial membrane invaginations, and transcellular pores within the EC body, a process orchestrated by cytoskeletal remodeling and ICAM-1 clustering[32]. The actin cytoskeleton within ECs plays a central role in regulating these processes, ensuring that barrier function is maintained during leukocyte passage[33].

After crossing the endothelial layer, leukocytes navigate the vascular basement membrane (BM) and the surrounding mural cell layer to reach the target tissue site[34]. The vascular BM, a dense extracellular matrix, contains regions of high and low protein expression, with low-expression regions serving as exit points for leukocyte transmigration, particularly for neutrophils[35]. Mural cells, activated by the inflammatory microenvironment, secrete chemokines to guide leukocytes through the venular wall and into inflamed tissues. Pericytes directly interact with neutrophils after they breach the endothelium, supporting subendothelial crawling and guiding their migration via adhesion molecules such as ICAM-1 and VCAM-1[36–39].

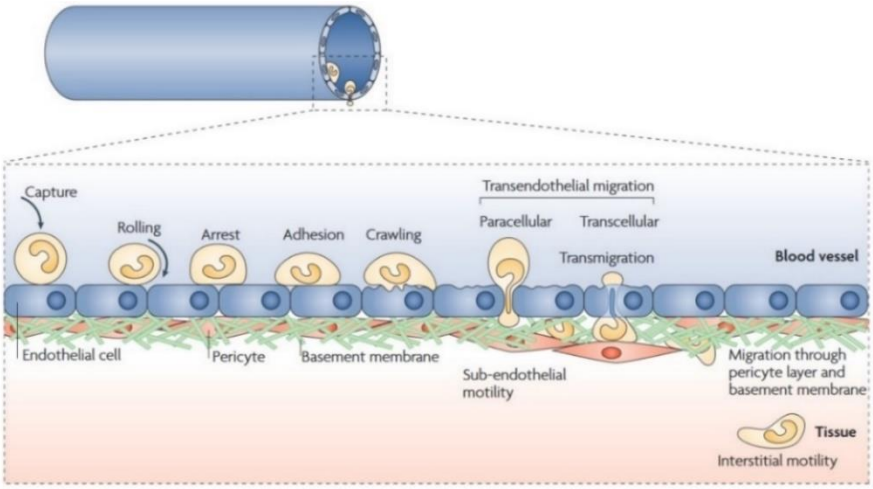

Figure 2. *Schematic overview of leukocyte adhesion, transendothelial migration (TEM) and migration through different components of post-capillary venular walls. This figure is adapted from Nourshargh et al. (2010) [34].*

3. Tissue-Resident Macrophages (TRMs)

3.1. Development and Diversity of TRMs

Tissue-resident macrophages (TRMs) are specialized immune cells that reside in various tissues throughout the body, where they play essential roles in maintaining homeostasis, supporting development, and regulating immune responses[40]. Many TRMs originate from yolk-sac-derived erythro-myeloid progenitors (EMPs), which emerge early during embryogenesis. These progenitors migrate to developing tissues, where they differentiate into macrophages[41]. Yolk-sac-derived macrophages play critical roles in organogenesis, angiogenesis, and establishing long-lived, self-renewing populations in organs such as brain, liver and lungs. Their specialization and functional adaptation are guided by tissue-specific signals[42,43]. As development progresses, fetal liver-derived monocytes, also originating from EMPs, contribute to certain TRM populations. These monocytes enter tissues during late embryogenesis and differentiate into macrophages that complement yolk-sac-derived populations[44].

The development of the adult hematopoietic system is based on distinct embryonic processes that lead to the emergence of hematopoietic stem cells (HSCs). Early hematopoiesis begins in the yolk sac, producing primitive blood cells that support early embryonic needs but without the capacity for long-term blood production. Definitive HSCs, which possess self-renewal and multi-lineage differentiation capacities, emerge later in development. These cells arise in intraembryonic regions such as the aorta-gonad-

mesonephros (AGM), placenta, and fetal liver, which act as key niches for their expansion and maturation. Definitive HSCs are responsible for establishing the lifelong hematopoietic system that sustains blood and immune cell production in adults. These HSCs migrate and colonize the bone marrow, which becomes the primary site of hematopoiesis in adults. This transition ensures a continuous supply of blood cells necessary for oxygen transport, immune defense, and tissue repair[45].

Macrophages derived from hematopoietic stem cells (HSCs) may supplement or replace yolk-sac-derived macrophages within tissues. In adulthood, HSC-derived monocytes from the bone marrow circulate in the bloodstream and enter tissues in response to injury or inflammation. These monocytes differentiate into macrophages at these sites, where they replenish or replace depleted embryonically derived populations and contribute to tissue repair and immune responses[46].

TRMs are a diverse, specialized population, with functions shaped by their tissue environment and specific locations within those tissues[47]. For instance, resident cardiac macrophages consist of a dominant population derived from embryonic yolk sac progenitors that self-renews in adulthood and a smaller subset from fetal liver precursors maintained by circulating monocytes. During disrupted homeostasis, monocyte-derived macrophages are recruited and can permanently replace embryonically derived resident populations[48].

3.2. Perivascular Macrophages (PVMs)

During vascular development, TRMs interact with ECs within angiogenic vascular niches. In this context, TRMs regulate EC behavior, while ECs modulate TRM function[49]. In many adult tissues, some TRMs establish close contacts with blood vessels and are referred to as perivascular macrophages (PVMs). Although there is no universally accepted definition of PVMs, they are often characterized by their proximity to the abluminal surface of blood vessels and their expression of tissue-specific markers, which vary across tissue sites[50,51].

PVMs perform critical functions at the interface between blood and tissue, including regulating vessel permeability[52,53], patrolling and clearing pathogens that transmigrate across the vasculature into tissues[54,55], and modulating immune responses to sustain tissue hemostasis[56–59]. However, PVMs can also contribute to pathological processes, driving uncontrolled inflammation in conditions such as autoimmune, degenerative, and tumorigenic diseases. Dysfunction in resident macrophages, whether caused by genetic or environmental factors, disrupts their regulatory roles, leading to compromised vascular integrity, impaired immune responses, and aberrant tissue remodeling. These diverse functions establish PVMs as central players in both maintaining physiological balance and driving disease progression[60–62].

4. Hereditary Hemorrhagic Telangiectasia (HHT)

4.1. Pathophysiology and Clinical Manifestations of HHT

Hereditary hemorrhagic telangiectasia (HHT), also known as Rendu-Osler-Weber syndrome, is an autosomal dominant vascular disorder primarily associated with mutations in genes encoding components of TGF-β signaling in ECs, which are critical for vascular development and integrity. HHT type 1 (HHT1) is caused by mutations in the *ENG* gene on chromosome 9, which encodes endoglin, while HHT type 2 (HHT2) results from mutations in the *ACVRL1* on chromosome 12, which encodes ALK1. Mutations in *SMAD4* have also been implicated in HHT phenotypes[63,64].

These genetic alterations disrupt normal angiogenesis, and mural cell recruitment to the endothelium, leading to fragile vessels and vascular malformations prone to bleeding[65–67]. Clinically, HHT is characterized by small telangiectasias in the skin and mucous membranes, as well as arteriovenous malformations (AVMs) in organs such as the brain, lungs, liver, and gastrointestinal tract[68,69]. AVMs, which are direct connections between arteries and veins without intervening capillaries, arise from vessel dilation and wall thinning[70]. Large AVMs can cause severe complications such as hypoxemia, stroke, heart failure, and fatal hemorrhage.

In HHT, mutations in *ENG* and *ACVRL1* result in loss of function, with haploinsufficiency identified as a key mechanism driving disease pathology. Physiological vessel development remains largely normal in HHT patients, and vascular lesions are localized to specific sites rather than being present throughout the body. The variability of clinical manifestations, even among patients with identical mutations, indicates that haplo-sufficiency of the remaining allele prevents widespread pathology. However, data from mouse models reveal that when the remaining allele is lost or its function is compromised by additional triggers, the risk of AVM formation increases significantly. These secondary triggers, collectively termed “second hits,” include epigenetic factors inducing biallelic somatic mutations and environmental factors such as pro-angiogenic stimuli, vascular injury, and inflammation[71–74]. Addressing these secondary factors alongside primary genetic mutations is critical for developing effective therapies that restore vessel stability and prevent disease progression. Recent therapeutic interventions targeting EC-mural cell interactions, angiogenic and inflammatory pathways have shown promise in stabilizing vessels and relieving HHT symptoms in patients, such as severe nosebleeds[75–80].

4.2. The Role of ENG in Innate Immune Regulation

Endoglin (ENG), a transmembrane glycoprotein, is expressed not only on ECs but also on innate immune cells, particularly monocytes and macrophages. During monocyte

differentiation from hematopoietic stem cells (HSCs), high levels of ENG are expressed[81]. While ENG is best known as an auxiliary receptor in the TGF-β receptor complex, it also mediates TGF-β-independent functions. Specifically, its arginine-glycine-aspartic acid (RGD) motif binds to integrins, facilitating integrin-mediated cell adhesion—a critical mechanism in inflammatory leukocyte recruitment. Studies have demonstrated that ENG interacts with leukocyte integrins, enhancing adhesion to ECs and promoting immune cell trafficking during inflammation. In endoglin-deficient mice, leukocyte extravasation in response to inflammatory stimuli is significantly reduced, highlighting the importance of ENG in immune cell trafficking in HHT[82,83].

Moreover, ENG regulates macrophage polarization into pro- inflammatory or anti-inflammatory subtypes[84]. In a mouse model, endoglin-deficient macrophages exhibit impaired phagocytic activity and reduced secretion of inflammatory cytokines, such as IL-1β and IL-6, in response to lipopolysaccharide (LPS) injection. These findings suggest that ENG plays a role in regulating the innate immune response[85]. In HHT patients, deficiencies in macrophage function may increase susceptibility to infections[86,87].

5. Vascular Inflammation and Disease Modelling *in vitro*

Animal models have significantly advanced our understanding of vascular disease mechanism and the underlying chronic or systemic inflammation. However, the physiological differences between mice and humans must be carefully considered when using mouse models to study human diseases. For instance, immune cell phenotypes, cytokine signaling pathways, and antigen presentation differ substantially between the two species, which can influence disease progression and affect the efficacy of therapeutic strategies in clinical trials[88,89]. Therefore, there is an urgent need for human-based models to study vascular pathologies and accurately assess disease mechanisms.

Access to patient-derived vascular cells is often limited, especially in the case of rare vascular diseases. While human cell lines have been widely used to study vascular pathology and inflammation, their prolonged culture often results in a loss of cellular specificity and function. Moreover, these cell lines lack the genetic background of patient-specific cells. To overcome these limitations, human induced pluripotent stem cells (hiPSCs) are becoming regarded as transformative tools for vascular disease modeling and drug discovery. This revolutionary technique allows reprogramming somatic cells into a pluripotent state using specific transcription factors[90]. hiPSCs derived from patients with disease-associated mutations can also be genetically engineered to correct or remove the gene variants, creating isogenic pairs for controlled comparisons. Patient-specific hiPSC lines provide virtually unlimited supply of cells for *in vitro* functional assays and drug testing applications[91].

hiPSCs have been successfully differentiated into vascular cell types, including ECs, vSMCs and pericytes[92–95], as well as immune cells such as monocytes and macrophages[96–98]. Despite their remarkable potential, challenges remain regarding the functional maturity of hiPSC-derived cells. Advanced *in vitro* platforms that provide multicellular environments with precise microenvironmental control offer a promising solution.

6. Organ-on-Chip Systems

Microfluidic chips have emerged as advanced *in vitro* tools for studying human physiology across various organ and tissue types. These systems typically consist of culture chambers connected by microchannels or semi-permeable barriers. Cells can be cultured on hydrogel surfaces or within hydrogel matrices, while the chambers can be perfused at controlled flow rates or subjected to mechanical deformations, such as stretching. Incorporating multiple cell types into these devices has enabled the study of organ-level processes, leading to the development of organ-on-chip (OoC) models, also known as microphysiological systems (MPS)[99]. OoC/MPS technologies offer new avenues for the study of human (patho)physiology that hold significant promise for translational research and preclinical drug screening.

6.1. Vessel-on-Chip

In the field of vascular biology, OoC technology offers innovative 3D vessel models that incorporate shear stress from fluid flow, supporting functional vascular output measurements in environments that better replicate physiological conditions compared to conventional 2D static models. These advancements provide more biologically accurate platforms for investigating vascular diseases[100–102].

A variety of techniques are employed to generate such platforms, each with unique advantages and limitations (Figure 3). The choice of technique, therefore, should align with specific research objectives[103]. Rectangular microchannels coated with ECs are among the simplest microfluidic platforms, often used to study EC responses to shear stress and their interactions with perfused leukocytes[104]. More advanced designs use adjacent microchannels separated by porous membranes to facilitate co-culture of ECs with tissue-specific cells, enabling studies on cell-cell communication[105]. Biomimetic hydrogels flanking the microchannels allow the modeling of leukocyte transmigration through a single EC layer into hydrogel compartments[106].

Studying interactions between ECs and mural cells is complex and requires combining 3D geometry and controlled fluid flow. Template-based strategies provide a versatile solution by generating 3D lumen structures within hydrogel matrices. These lumens are created by inserting a sacrificial rod into the unpolymerized hydrogel, which is removed post-

polymerization, leaving cylindrical channels that can be seeded with ECs to form an endothelial barrier[107]. Alternative techniques such as viscous finger patterning offer a more robust method to create hollow tubes within hydrogels[108]. More advanced laser ablation methods allow precise patterning of complex lumen structures, including capillary-sized lumens, with greater control over the geometry[109]. These systems enable ECs to attach to hydrogel interfaces while mural cells embedded within the matrix interact directly, creating a membrane-free environment well-suited to studying vessel inflammation and leukocyte transmigration.

Self-assembling VoC models leverage the inherent vasculogenic ability of ECs to form vascular networks autonomously, closely mimicking natural processes. These models allow the integration of mural cells, enhancing the relevance of the vascular niche *in vitro*[110]. However, controlling the resulting structures can be challenging, leading to variability in network formation.

Bioprinting represents another cutting-edge approach, enabling the layer-by-layer deposition of bioinks—composed of living cells, biomaterials, and growth factors—to construct complex vascular networks. This technique can also be used to fabricate vascular networks in engineered tissue constructs[111].

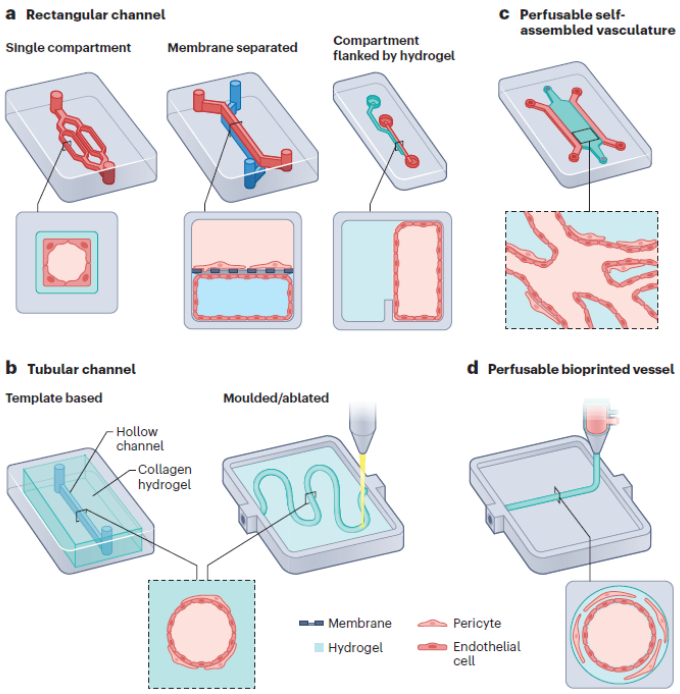

Figure 3. *Current microfluidic and VoC techniques to develop in vitro vessel models. This figure is adapted from Nahon et al. (2024)* [112].

7. Aim and Scope of This Thesis

Over the past decade, various advanced *in vitro* models of human vasculature have been developed. However, most of these models fall short in replicating the complex vascular microenvironment, particularly in the context of inflammation modeling and leukocyte recruitment. Moreover, modeling complex vascular diseases such as HHT1 remains challenging, as it requires integrating genetic factors and environmental triggers, such as inflammation and shear stress, that underlie disease pathology. In this thesis we aimed to develop VoC models that better capture the intricate dynamics of vascular inflammation, leukocyte transmigration, and microvascular development in health and disease. Specifically, we generated two VoC models incorporating hiPSC-derived ECs, mural cells, monocytes and macrophages to enhance their complexity and relevance to physiological conditions.

In **Chapter 2**, we describe a robust protocol for generating single-lumen-based VoC models incorporating hiPSC-derived ECs and mural cells. This model was designed to investigate vascular barrier function and set the foundation for further studies.

In **Chapter 3**, this VoC model was used to study inflammatory responses, with a focus on leukocyte transendothelial migration (TEM). hiPSC-derived monocytes were perfused under physiological shear stress to assess their interactions with the vascular barrier and the contribution of mural cells to this process. Additionally, we evaluated the functionality of hiPSC-monocytes derived from HHT1 patients, focusing on their TEM capacity under inflammatory conditions.

In **Chapter 4**, we expanded our focus to modeling HHT1 disease pathology using a self-assembling VoC model. Specifically, we investigated the role of inflammatory triggers in driving the vascular abnormalities associated with HHT1.

In **Chapter 5**, we aimed to enhance the complexity of the microvascular environment by incorporating hiPSC-derived macrophages into the self-assembly VoC model. This model was developed to mimic the phenotype of perivascular macrophages and to study their roles of in vascular function during health and inflammation.

In **Chapter 6**, we reviewed recent advances in generating functional vascular networks within vascularized human organoid models using microfluidic VoC strategies. These strategies aim to create more complex and physiologically mature human tissue models.

Finally, in **Chapter 7**, the findings of this thesis are discussed, along with its limitations and broader implications for vascular disease modeling. A future outlook is provided for advancing VoC platforms using patient-derived hiPSCs for drug development and translational research.

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