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Regulating Lymphatic Vasculature in Fibrosis: Understanding the Biology to Improve the Modeling

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Fibrosis occurs in many chronic diseases with lymphatic vascular insufficiency (e.g., kidney disease, tumors, and lymphedema). New lymphatic capillary growth can be triggered by fibrosis-related tissue stiffening and soluble factors, but questions remain for how related biomechanical, biophysical, and biochemical cues affect lymphatic vascular growth and function. The current preclinical standard for studying lymphatics is animal modeling, but in vitro and in vivo outcomes often do not align. In vitro models can also be limited in their ability to separate vascular growth and function as individual outcomes, and fibrosis is not traditionally included in model design. Tissue engineering provides an opportunity to address in vitro limitations and mimic microenvironmental features that impact lymphatic vasculature. This review discusses fibrosis-related lymphatic vascular growth and function in disease and the current state of in vitro lymphatic vascular models while highlighting relevant knowledge gaps. Additional insights into the future of in vitro lymphatic vascular models demonstrate how prioritizing fibrosis alongside lymphatics will help capture the complexity and dynamics of lymphatics in disease. Overall, this review aims to emphasize that an advanced understanding of lymphatics within a fibrotic disease—enabled through more accurate preclinical modeling—will significantly impact therapeutic development toward restoring lymphatic vessel growth and function in patients.

1. Introduction

Lymphatic vascular networks are found throughout the body, and they maintain interstitial homeostasis by serving as a drainage system for interstitial tissue fluid, cells, and macromolecules (e.g., proteins, lipids) that accumulate throughout the body.[1] These networks are comprised of lymphatic vessels that form during embryonic development and assemble into a vascular hierarchy that transitions from initial lymphatics (or capillaries) to pre-collecting lymphatics and larger collecting lymphatics.[2] Historically, lymphatics were viewed as a passive vascular network for toxin removal, immune cell trafficking, and dietary lipid transport. However, evidence over the years has shown that lymphatic vasculature plays a more active role in physiological and pathophysiological conditions.[3,4] Moreover, the diverse origins of lymphatic vessels produce organ- and disease-specific vascular growth, structures, and functions that put lymphatic vasculature in the spotlight as a potential therapeutic target,[4,5] especially for diseases or conditions with significant lymphatic involvement but no effective curative solutions.

Lymphangiogenesis—new vessel growth (or sprouting) from existing lymphatic vessels—is a key participant in many natural and pathological processes in adult tissues (e.g., wound healing, inflammation, tumor metastasis, fibrosis).[6] As lymphatic capillaries grow and branch, they form dense vascular networks to increase the overall surface area available for interstitial fluid uptake. Therefore, barrier function (measured by vessel permeability) that controls fluid movement across the vessel wall is the other key factor in dictating overall vascular sufficiency. Under certain pathological conditions, the tissue microenvironment surrounding lymphatic vasculature experiences significant changes that alter vessel growth and function.[7] Fibrosis—excess extracellular matrix (ECM) deposition and scarring of connective tissue—is a common occurrence across numerous pathological conditions that also involve significant amount of lymphatic dysregulation (e.g., chronic kidney disease, lymphedema, cardiac fibrosis, cancerous tumors).[8–13] Inflammation often works in tandem with fibrosis in many conditions to create a biophysical (e.g., ECM stiffening), biomechanical (e.g., increased interstitial pressure), and biochemical (e.g., pro-lymphangiogenic growth factors, inflammatory cytokines) environment that changes with time and perpetuates the disease state.[8,14–18] These changes not only influence lymphatic endothelial cell (LEC) behavior (i.e., proliferation, migration) and cell–cell junction integrity,[2,19,20] but they also affect stromal cell populations (e.g., fibroblasts, immune cells) that interact with LECs through paracrine signaling. Moreover, the altered signaling leads to vascular outcomes that deviate from the norm and significantly impact patient health. For example, an insufficient

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amount of functional lymphatic vessels can lead to fluid build-up in tissues (e.g., cardiac edema, lymphedema),[12,21–25] while hyper-sprouting of leaky capillaries (low barrier function) can enable tumor metastasis.[26–28] In chronic kidney disease with renal fibrosis, increased lymphangiogenesis helps drain excess fluid, but increased recruitment of dendritic cells to the lymph nodes combined with leaky vessels disrupts the immune response.[10,13]

Since inflammation and tissue stiffening can trigger lymphangiogenesis and alter barrier function,[14,16,20,29,30] studies on lymphatic vasculature should consider each of these factors when developing therapeutic strategies. Currently, there are differences in treatment approaches based on the type of lymphatic dysregulation. When fibrosis is involved, lymphatic vascular growth is often the primary therapeutic target to reverse or suppress further fibrosis development and disease progression.[1,13,21,23,30–32] For example, tumors and chronic kidney fibrosis are treated with anti-lymphangiogenic therapies (e.g., anti-VEGFR therapy) to counteract increased lymphatic vascular growth. On the other hand, lymphedema treatments promote lymphangiogenesis (e.g., VEGF-A therapy) because of insufficient vasculature.[13,21,23,31,33,34] However, cancer patients with secondary lymphedema cannot be treated with pro-lymphangiogenic therapies due to the risk of increasing peritumoral lymphatic vasculature that could promote metastasis.[35] Collectively, these approaches only address lymphatic growth and not function, likely due, in part, to the conflation of lymphangiogenesis with lymphatic vascular function (i.e., vascular permeability, valve function).[1,3,4]

There are also knowledge gaps surrounding fibrosis-mediated drivers of lymphatic vascular growth and function in disease, as well as temporal aspects of biochemical and biophysical signaling that vary across stages of fibrotic progression (early vs late). Nevertheless, without the appropriate tools and techniques to investigate these knowledge gaps, therapeutic development will remain stagnant. Currently, in vivo studies of lymphatics and disease are more common than in vitro, but the focus of both in vivo and in vitro models of lymphatic biology has largely been on development. This research focus has led to a better understanding of early stages of lymphatics during embryonic development, along with relationships to postnatal and pathological lymphatics (Reviewed[4,36–38]). However, pathological lymphatics still requires more attention in the in vitro modeling space. Therefore, we focus this review on disease states that impact lymphatic vascular growth and function rather than lymphatics in developmental stages to set the stage for next-generation modeling of pathological lymphatic vasculature.

After providing an overview of lymphatic vascular structure and function, we establish the current understanding of the interplay between lymphatic vasculature (primarily lymphatic capillaries) and the fibrotic microenvironment within the context of mechanoregulation and lymphatic vascular behaviors and responses observed in early- and late-stage fibrosis. We then delve into how tissue engineering and microfabrication approaches are being used to recreate lymphatic vasculature and the surrounding microenvironment for improved pre-clinical modeling in vitro. Even though a great deal of in vivo animal work has been done to study lymphatic vasculature, in vitro tissue-engineered models enable studies to systematically investigate key interactions and mechanisms that drive lymphatic vessel growth, function, and dysregulation. Finally, we present future research directions and opportunities in in vitro modeling and lymphatic tissue engineering research that can be used to increase understanding of lymphatic biology and pathological lymphatics and enhance therapeutic development.

2. Lymphatic Vascular Structure, Growth, and Function

2.1. Origins and Structure

Lymphatic vasculature is comprised of a branched vascular network that begins as blind-ended initial lymphatics/capillaries that connect to larger pre-collecting and collecting vessels (**Figure 1**). The larger vessels transport lymph fluid through lymph nodes that filter the fluid and return it to circulation.[39–41] Lymphatic vessels form during embryonic development and most arise from transdifferentiated venous endothelial cells that express prospero-related homobox 1 (Prox1; transcription factor). Multiple comprehensive review articles have been published to highlight new insights in our understanding of lymphatics in early development[36–38,42–44] and studies within the last decade have revealed organ-specific lineages that deviate from the typical venous origins (Reviewed by Petrova and Koh, 2018).[4] For instance, lumbar and cardiac lymphatic vessels primarily form from coalescing Tie2-lineage- nonvenous LEC progenitors,[45,46] while thoracic, cervical, and skin lymphatic vessels form from lymphangiogenic sprouting of Tie2-lineage$^+$ LEC progenitors.[45] Differences across organs also extend to signaling downstream of established lymphatic vessel regulators such as vascular endothelial growth factor (VEGF)-C/ vascular endothelial growth factor receptor (VEGFR)-3 and Prox1,[47] which may have future implications in engineering organ-specific LECs for regeneration. Moreover, organ-specificity in lymphatic vasculature may contribute to disease-specific lymphatic vasculature and related behaviors that influence therapeutic strategies.

Even with organ-specificity, many structural features of lymphatic vessels are conserved between organs, and all LECs express Prox1, VEGFR3, and lymphatic vessel endothelial receptor 1 (LYVE-1).[48] However, some features vary along the lymphatic vascular hierarchy. Lymphatic capillaries (diameter: 10–75 μm) are comprised solely of LECs and are not surrounded by a continuous basement membrane or mural (support) cells. Instead, the capillaries have discontinuous "button-like" junctions between oak leaf-shaped LECs[40] to provide points of entry to transport interstitial lymph fluid containing macromolecules (e.g., lipids, proteins) and cells (e.g., immune) from tissues across capillary walls via transmural flow.[49,50] Lymph fluid is transported from the capillaries to the pre-collecting vessels and collecting vessels, but unlike lymphatic capillaries, LECs in pre-collecting and collecting lymphatic vessels have continuous cell–cell junctions (i.e., zippers). These larger vessels are also covered by basement membrane and smooth muscle cells (i.e., mural cells) on the basal side of LECs,[51] which makes them less permeable than capillaries. Smooth muscle cell contractions—along with compressions and pulsation from

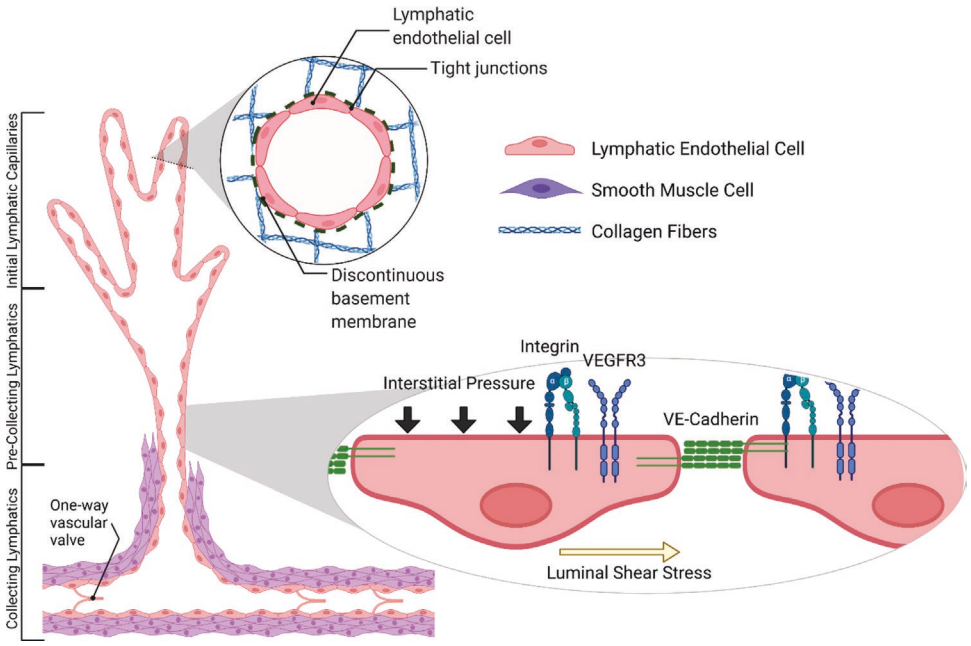

Figure 1. Schematic representation of lymphatic vascular hierarchy (left). Lymphatic endothelial cells (LEC), surrounded by a discontinuous basement membrane, form lymphatic capillaries that drain interstitial fluid into the pre-collecting lymphatics before reaching collecting lymphatics that are covered by a continuous basement membrane and peripheral smooth muscle cells. A pressure gradient is formed in collecting lymphatics via the one-way vascular valves. Lymphatic capillaries interface directly with the interstitial ECM of the surrounding tissue (top). The LECs experience biomechanical inputs such as interstitial pressure and luminal shear stress. Cell surface receptors such as integrins and VEGFR3 are involved with cell–ECM interactions and growth factor signaling, respectively, and LECs retain structure and barrier function via cell–cell adhesion molecules such as VE-cadherin. Created with BioRender.com.

surrounding skeletal muscle and arteries—help move lymph fluid through the pre-collecting and collecting vessels, and one-way valves within the vessels support unidirectional lymph fluid flow toward the lymph nodes.

2.2. Lymphatic Vascular Growth

Lymphatic endothelial cell proliferation and migration are critical for lymphangiogenesis and both processes are regulated, in part, by binding between VEGF-C/D (secreted by LECs and stromal cells) and VEGFR3 (highly expressed on the LEC surface).[6] Additional growth factors such as fibroblast growth factor (FGF)[52] and platelet-derived growth factor (PDGF)[53] are also involved in lymphangiogenesis but are likely secondary drivers after VEGF-C/D. Interactions between LECs and the surrounding ECM via integrins and other cell surface proteins also regulate lymphangiogenesis via VEGFR3 activation as these molecules sense and respond to biophysical or biomechanical stimuli in the tissue microenvironment.[54,55] Upon VEGFR3 activation, LECs form protrusions that search for biophysical or biochemical cues to guide migration.[54] As these tip cells migrate, proliferating LECs that are also stimulated by VEGFR3 activation facilitate capillary elongation. The elongating vessels eventually stabilize as button-like junctions form between LECs to establish barrier function and control fluid transport across the capillary wall.[56] These steps lead to the formation of normal lymphatic vasculature, as long as growth factor, biophysical, and biomechanical signaling is maintained at normal levels. However, when there is an imbalance, disruptions to lymphangiogenesis and vascular function occur.

2.3. Lymphatic Vascular Functions

Beyond transporting lymph fluid, lymphatic vasculature has numerous biological functions that are active in homeostasis and disease (Reviewed by Petrova and Koh, 2020).[57] The system's primary functions are fluid level balance, waste removal, immune system regulation and transport, and dietary fat transport and absorption in the intestine. However, functions may also change with changes to biological demands or disease states. For example, pre-existing and newly formed lymphatic capillaries can be found at the tumor periphery and in the intramural space of various solid tumors.[16,26,27,58] These capillaries no longer function to maintain homeostasis, and instead they enable cancer and immune cell entry into lymphatic vasculature to facilitate metastasis, immune cell trafficking, and disease progression. Biochemical signaling also factors into lymphatic vascular function as the variable molecular expression on lymphatic capillaries and along the lymphatic vessel hierarchy influence functions and outcomes.[48]

Button-like junctions between LECs in lymphatic capillaries and zipper-like junctions in pre-collecting and collecting vessels are the major gatekeepers for controlling barrier function (i.e., vascular permeability) for transmural fluid transport (Reviewed by Baluk and McDonald, 2022).[59] Under normal conditions, button junctions function as "primary valves" and are typically regulated by interstitial pressure to control fluid entry into capillaries.[60,61] As pressure increases, lymphatic capillary lumens stretch to accommodate increased flow and collapse as pressure drops.[91,110] More dramatic pressure changes can occur in disease (e.g., solid tumors), but overstretching the lumens can permanently damage barrier function.[111] Other regulators

of barrier function include molecules such as Angiopoietin-2, VEGF-A/VEGFR2, and VEGF-C/VEGFR3 (Reviewed by Zhang et al., 2020).[2] Angiopoietin-2 is important for junction maturation in lymphatic development and likely plays a role in controlling vascular endothelial (VE)-cadherin phosphorylation for button junction formation.[62] On the other hand, VEGFR2 signaling promotes zipper junction formation in intestinal capillaries that decreases lipoprotein uptake.[63] Interestingly, while VEGF-C/VEGFR3 signaling is critical for LEC proliferation, differentiation, migration, and apoptosis, there is less agreement about whether VEGF-C/VEGFR3 regulates LEC junctions. Some studies suggest that VEGF-C exposure decreases barrier integrity,[64,65] while others say that VEGF-C/VEGFR3 signaling has no effect on junctions and barrier function.[63]

When considering immune system regulation, differences emerge between the types of lymphatic vessels involved. For example, lymphatic capillaries express chemokine (C–C motif) ligand 21 (CCL21) to attract $CCR7^+$ dendritic cells and LYVE-1 to bind hyaluronic acid (HA), a glycosaminoglycan in the ECM. Conversely, collecting vessels have lower expression of molecules that are more active in lymphangiogenesis (e.g., VEGFR3) and immune cell trafficking (e.g., CCL21, LYVE-1).[66–68] In capillaries, LYVE-1 on LECs facilitates HA turnover within the ECM surrounding lymphatic vessels,[69] and as HA is taken up by LECs, it is transported to lymph fluid within the lymphatic vessels. LYVE-1 also binds to HA in the glycocalyx of dendritic cells to aid in their trafficking through lymphatic vessels.[70,71] In the intestine, lymphatic vessels specifically play a role in fat absorption. Fat-soluble vitamins and dietary lipids are absorbed by enterocytes and enter the intestinal lymphatics through intestinal lymphatic capillaries (i.e., lacteals).[72] Intestinal lymphatics also experience continuous, tightly regulated VEGFR3 signaling as LEC proliferation and lymphangiogenesis are ongoing processes that are likely sustained by high mechanical and biochemical stresses within the intestine.[73]

3. Fibrosis, Inflammation, and Lymphatics

One source of microenvironmental change that impacts lymphatic vascular growth and function is fibrosis, which involves excess ECM deposition (e.g., collagens, fibronectin) and connective tissue remodeling via myofibroblast activation.[17,74] Collagens within the ECM also undergo enzymatic crosslinking via lysyl oxidase, lysyl oxidase like-enzymes 1-4, peroxidasin, and transglutaminase 2 that strengthens and linearizes collagen fibers.[75] The combination of ECM accumulation and crosslinking leads to overall tissue stiffening, altered ECM organization, and degradation resistance, which has a significant impact on cell responses. Fibrosis can occur in nearly every tissue of the body with some degree of organ specificity,[9,11] that can be dictated, in part, by the diversity in myofibroblast sources and subpopulations (e.g., hepatic stellate cells, resident kidney fibroblasts) across organs. Varied immune cell infiltration also occurs in response to acute injury, and the resulting inflammatory response promotes myofibroblast activation and produces inflammatory and pro-fibrotic soluble factors (e.g., growth factors, cytokines, proteolytic enzymes) from immune cells (e.g., macrophages, neutrophils).[76,77]

In chronic kidney fibrosis, kidney cell damage induces an inflammatory response that recruits inflammatory cells and activates resident fibroblasts and myofibroblasts.[78,79] Macrophages within the kidney secrete VEGF-C/D to promote lymphangiogenesis and sustain fibrosis,[80] and neutrophils stimulate LEC proliferation and lymphatic growth by releasing VEGF-C/D and increasing the bioavailability of ECM-bound VEGF-A.[81] In cardiac fibrosis that arises from acute myocardial injury, initial inflammatory responses, which include an influx of immune cells (e.g., macrophages, neutrophils) and increased expression of inflammatory cytokines, stimulate cardiac fibroblast activation.[18] Without proper fluid drainage from damaged capillaries and altered pre-collecting vessels, interstitial fluid pressure increases and puts further mechanical strain on cardiac fibroblasts. Interestingly, studies over the years reveal that idiopathic pulmonary fibrosis involves very little inflammation unlike other fibrotic diseases.[77] Lymphatic capillaries in pulmonary fibrosis experience abnormal remodeling in early disease stages when mural cells are recruited to vasculature that typically lack mural cells (i.e., capillaries, pre-collecting vessels).[82] Fibroblasts also aggregate around pulmonary lymphatic vessels in response to higher accumulation of HA that reduces lymph fluid transport across the vessel walls and further contributes to fibrosis.[32,83]

Although fibrosis and inflammation are not the only causes of lymphatic vascular dysregulation, they are a major source in several disease states. In chronic (late-stage) fibrotic conditions (e.g., chronic kidney fibrosis, cardiac fibrosis, idiopathic pulmonary fibrosis), the myofibroblast response goes unchecked and reaches a pathological level[84,85] that often results in tissue stiffening within the microenvironment.[86–89] Together with soluble factors, tissue stiffening produces pathological vasculature with altered lymph fluid flow characteristics and transmural transport properties that maintain or worsen the disease state.[8–11,76,77,80] Moreover, increased lymphatic vascularization can promote fibrosis via a positive feedback loop,[12,23,83,90–92] and when rapid lymphangiogenesis occurs, vascular permeability (i.e., barrier function) can increase[21,23,93] and produce leaky, nonfunctional vessels.[29,87,94] Conversely, there are disease states with significant fibrosis (e.g., secondary lymphedema, myocardial edema) and insufficient lymphatic vasculature (i.e., inadequate growth, low number of vessels, low vessel function).[21,32] Without sufficient functioning lymphatic vasculature, tissues often swell due to the ineffective transport of excess fluids back into the blood stream, which causes pain and other health issues for patients. For these and other conditions that significantly impact lymphatic vasculature, improved therapeutic targeting is necessary to achieve a curative outcome rather than merely manage symptoms.

4. Mechanoregulation in Lymphatic Vasculature

The tissue surrounding lymphatic vasculature produces a variety of dynamic biomechanical (i.e., flow, pressure, and muscle contractility) and biophysical (e.g., ECM stiffness) stimuli that act directly on the vessels. Structural differences across the vascular hierarchy also influence mechanical signaling in the tissue microenvironment as fluid moves through

and around lymphatic vasculature. Mechanosensitive molecules and intracellular mechanotransduction signaling pathways that are activated by mechanical inputs are responsible for how LECs and stromal cells sense and respond to these dynamic changes (Reviewed by Geng et al., 2021[95]). Lymphatic capillaries primarily experience transmural flow across the vessel wall via their discontinuous button-like junctions, which differs from higher magnitude laminar flow, luminal shear, oscillatory shear, and interstitial pressure experienced by LECs in larger collecting vessels (Figure 1).[50] However, lymphatic capillaries are still sensitive to changes in fluid shear stress, cyclic stretch, and interstitial fluid pressure, as well as stiffness in the surrounding ECM.

The downstream mechanoregulation events that affect growth and function in lymphatic capillaries are heavily tied to cell–cell and cell–ECM adhesion molecules expressed in LECs and some stromal cell populations. Cell–cell adhesion molecules between LECs, such as platelet endothelial cell adhesion molecule-1 (PECAM-1) and VE-cadherin, reorganize and downregulate in response to transmural flow, which directly impacts vessel integrity and function via changes in vessel permeability.[60] Since lymphatic capillaries do not have a basement membrane, their capacity to sense biophysical and biomechanical changes in the microenvironment is enabled by direct interactions between LECs and the underlying interstitial ECM through adhesion molecules like elastin microfibril interfacer 1 (EMILIN1)[96,97] and integrins.[60] During lymphangiogenesis, EMILIN1 attaches to elastin and fibulin-5 within the ECM, while integrins attach to fibronectin ($\alpha9\beta1$, $\alpha4\beta1$, $\alpha5\beta1$) and types I and IV collagens ($\alpha1\beta1$ and $\alpha2\beta1$).[60] The collagen fibers that are anchored to LECs also help to open and close capillary lumens as interstitial pressure changes in the tissues.[91,110] In addition to cell–ECM adhesion, EMILIN1 helps maintain lymphatic capillary integrity[97] and interacts with $\alpha9\beta1$ and $\alpha4\beta1$ integrins to regulate lymphangiogenesis.[98] In fact, studies with EMILIN1-deficient mice showed enlarged visceral and dermal lymphatics with a decreased capacity to respond to changes in interstitial fluid pressure and appropriately regulate lymphatic drainage.[97] This effect on lymphatic vasculature was not observed in developing lymphatics, which suggests that EMILIN1 expression is more critical for postnatal vascular maintenance and in disease.

Mechanosensitive molecules and pathways transduce mechanical inputs into biochemical signals in normal and pathological conditions.[60,96,97] For example, VEGFR3 and VEGF-C/D signaling are part of a mechanosensitive signaling pathway that regulates developmental and postnatal lymphangiogenesis. Shear forces and stretch activate VEGFR3, which binds soluble VEGF-C and VEGF-D.[40,55,99] These binding events lead to downstream phosphorylation of serine kinases AKT and ERK, which increases LEC proliferation, migration, and overall cell health.[33,100] The mechanical microenvironment of lymphatic vessels also impacts dendritic cell trafficking, as production of CCL21 increases with flow in LECs.[101] Transcription factors within LECs are also sensitive to changes in the tissue microenvironment, with GATA binding protein 2 (GATA2), yes-associated protein (YAP), and transcriptional co-activator with PDZ-binding motif (TAZ) being responsive to changes in tissue stiffness, vessel stretch, fluid shear, and growth factor signaling.[49,50,68,102–105] Yet, the responses are often context-dependent (e.g., type of mechanical input; in vitro versus in vivo; vessel type) and not directly correlated with high or low mechanical stimuli. For example, GATA2 transcription is upregulated when LECs are exposed to softer/lower ECM stiffness substrates in vitro[104] and higher oscillatory shear in vivo.[105,106] However, despite showing mechanotransduction relationships with GATA2 expression, the responses observed in these studies differ with the type of vessel and are only comparable to behaviors observed in early lymphatic development and not in disease. Both YAP and TAZ also respond to numerous types of biomechanical stimuli, including oscillatory shear stress[49,68] and ECM stiffness.[104,107] However, when YAP and TAZ are mechanically activated, their ability to positively or negatively regulate lymphangiogenesis depends on their downstream targets and whether activation occurs in fibroblasts or LECs. Specifically, stiffness-mediated YAP/TAZ activation in fibroblasts increases VEGF-C production and promotes lymphatic growth,[108,109] which is in contrast to LECs where YAP/TAZ activation downregulates Prox1 and leads to reduced VEGFR3 expression and decreased LEC sensitivity to VEGF-C.[110–113]

Overall, these and other mechanotransduction events can play significant roles in regulating lymphatic growth and function, primarily through LEC proliferation and migration. Much is known about the effects of biomechanical inputs (e.g., flow, stretch, interstitial pressure), because they have historically dominated the lymphatic narrative.[5] However, gaps still remain in the field of lymphatic vascular mechanobiology, because less attention has been placed on investigating the contributions of ECM stiffness, and much of the research to date focuses on lymphatics in embryonic development rather than disease.[60,96,97] As more studies emerge to focus on ECM stiffness and lymphatics under normal and pathological conditions,[104,107] the field will have a deeper, more comprehensive understanding of lymphatic mechanobiology. Moreover, the knowledge gained about mechanosensitive and mechanotransduction molecular activity could inform the selection of therapeutic targets to restore lymphatic vascular growth and function in several pathological conditions.

5. Dysregulated Lymphatic Vascular Growth and Function

Under normal homeostatic conditions, LEC proliferation and migration stay balanced to promote proper vascular growth/sprouting for vessel extension and stable network formation across the lymphatic vessel hierarchy. Capillaries should also establish discontinuous cell–cell junctions for controlled lymphatic capillary permeability (i.e., barrier function) (**Figure 2**A).[6,21,23] However, once proliferation and migration are no longer balanced, lymphatic vessel number, density, size, function, etc. can change to varying degrees of impact. For example, insufficient vasculature (e.g., low number, size) results in poor lymph fluid drainage from interstitial tissues. Drainage is also inhibited if capillary permeability is too high (low barrier function), because a fluid pressure gradient cannot form to open the capillary lumen.[114–116] Alternatively, if capillary permeability is too low (high barrier function), fluid cannot

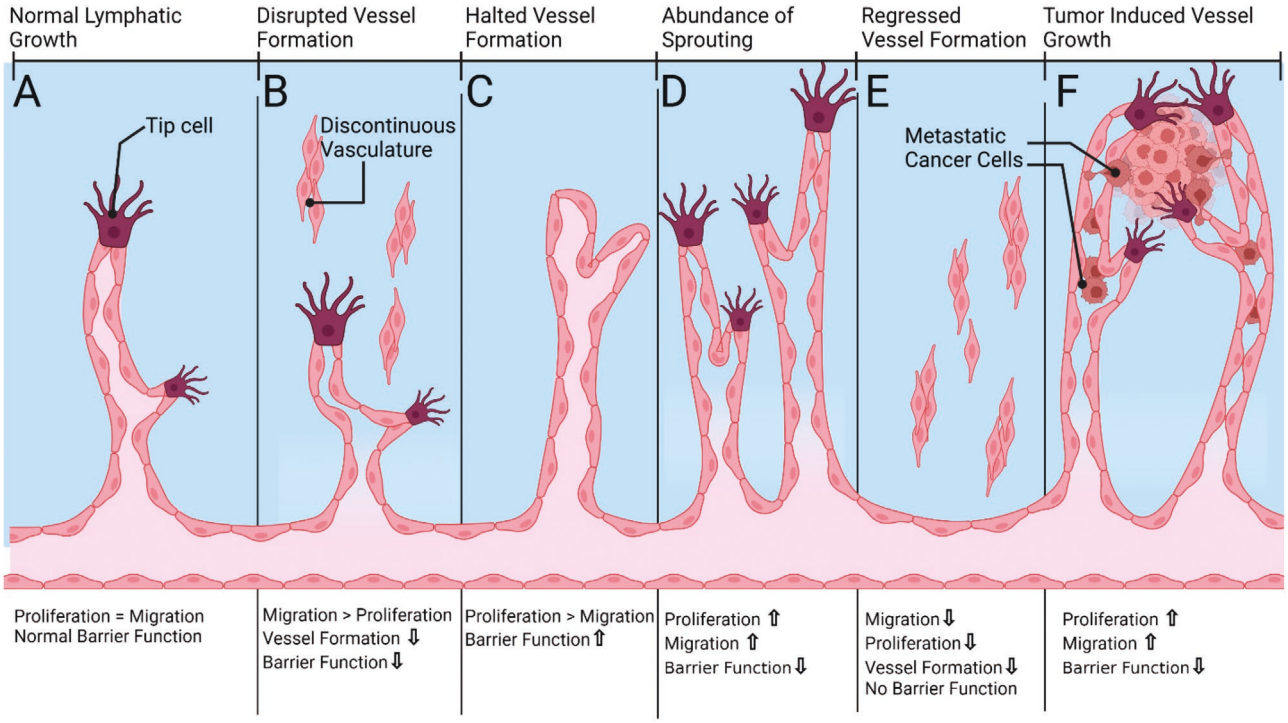

Figure 2. Schematic describing different modes of lymphatic capillary growth and function that can occur under different microenvironmental conditions or disease states. Effects on LEC proliferation, migration, and subsequent vessel formation are the primary focus. A) Normal lymphatic growth produces sprouting vessels with balanced LEC proliferation and migration with controlled barrier function. B) Migration outpaces proliferation and results in disrupted vessel formation and discontinuous vasculature. C) Proliferation outpaces migration and results in vessels with high barrier function. D) Increased sprouting of vessels (higher density) with low barrier function can occur during early-stage fibrosis. E) Vessel regression and discontinuous vasculature can occur during late-stage fibrosis. F) Tumor microenvironments can combine chronic fibrosis with continuous biochemical signaling to produce and maintain abundant vasculature with low barrier function, which allows intravasation and metastasis of cancer cells. (Up and Down arrows represent increased or decreased activity relative to the normal state). Created with BioRender.com.

enter the capillaries. These permeability changes can result from an inability to form proper junctions, decreased capacity to regulate junction integrity, or transformation from button to zipper junctions. Overall, different factors, including altered biochemical and biophysical signaling associated with fibrosis, disrupt homeostasis to produce lymphatic vasculature across a spectrum of vascular growth, morphologies, and functions (Figure 2B–F). These variations change with time and depend on microenvironmental and/or pathological changes and whether signaling occurs in LECs or stromal cells.

Changes within the tissue microenvironment can result in either discontinuous lymphatic vasculature or halted vascular growth (Figure 2B,C) with low and high barrier function, respectively. In discontinuous vasculature (Figure 2B), LECs migrate into the tissue but ultimately do not assemble to form stable vessels with intact cell–cell junctions.[30] An in vitro study identified a role for inflammation in regulating barrier function when it showed how inflammatory cytokines (e.g., TNF-α, IL-6, IL-1β, INF-γ, LPS) from stromal cells increase LEC cell–cell permeability, in part by reducing VE-cadherin expression.[19] In addition to cell–cell adhesion, barrier function in lymphatic capillaries is also moderated by actin cytoskeleton activation and stability in LECs that can be positively affected by the upregulation of FOXC2 (transcription factor), VE-cadherin, and Rac1 (Rho GTPase).[5,19,117] When considering halted vasculature (Figure 2C), increases in TGF-β1 secretion during fibrosis cause increased ECM stiffness and density due to fibroblast-mediated ECM deposition, which can inhibit lymphatic capillary growth.[12,23,93] In addition, LEC migration and capillary growth can be compromised by high YAP/TAZ expression in LECs that leads to low expression of Prox1.[107] Low Prox1 leads to low VEGFR3 availability, which decreases VEGF-C/D binding and may increase VE-cadherin expression (i.e., barrier integrity).[65]

Abundant lymphatic capillary sprouting beyond what is considered to be "normal" growth (Figure 2D) can occur in a number of ways. One of the most common mechanisms for abundant or hyper-sprouting is increased VEGF-C/VEGFR3 signaling in stromal cells and LECs that increases LEC proliferation and migration. Increased TGF-β1 production in fibroblasts during fibrosis stimulates VEGF-C secretion from immune cells to promote lymphatic capillary growth,[118] which then creates a positive feedback loop as newly formed vasculature recruit more immune cells for paracrine signaling.[21,119,120] Stiffening within the ECM can also activate YAP/TAZ in fibroblasts to increase VEGF-C secretion.[108,109] In LECs, higher Prox1 expression correlates with increased VEGFR3 expression and VEGF-C sensitivity to promote LEC proliferation and subsequent capillary growth.[107] Higher Prox1 also increases matrix metalloproteinase (MMP)-14 expression and subsequent ECM degradation to enhance LEC migration. Barrier function may also be compromised with hyper-sprouting. For example, in chronic inflammation, significant lymphatic capillary sprouting

often occurs, and inflammation in airway lymphatics was shown to promote button-to-zipper transformation of intercellular junctions in newly formed capillaries.[20] Since zipper functions are tighter than button junctions, the result is decreased vessel permeability. Hyper-sprouting can also lead to poorly developed vasculature with very low barrier function (i.e., leaky vessels) that eventually regresses into discontinuous, nonfunctional vasculature with few cell–cell adhesions and no measurable barrier function (Figure 2E).

Low barrier function is also a factor in diseases like cancer where overactive lymphatic capillary growth results in many highly permeable lymphatic capillaries that allow an influx of cells (e.g., metastasizing cancer cells, immune) and macromolecules into the leaky vasculature (Figure 2F). Tumors present unique microenvironments that promote lymphatic capillary growth in the peritumoral space through sustained pro-lymphangiogenic and pro-inflammatory signaling (e.g., VEGF-C, hypoxia inducible factor (HIF)-1α).[26–28] We have already established that LECs proliferate and migrate when VEGF-C binds to VEGFR3, but M2 polarized macrophages (pro-fibrotic, anti-inflammatory) within the tumor microenvironment secrete even more VEGF-C to increase lymphatic capillary growth.[121] Increased binding of VEGF-C to VEGFR3 also decreases VE-cadherin expression in LECs, which compromises cell–cell junctions between LECs and increases capillary permeability to enable metastasis.[65]

As we describe lymphatic vascular outcomes that result from altered biophysical and biochemical microenvironments, **Table 1** provides an overview of relevant diseases or conditions with dysregulation that is related to fibrosis. We form general categories that highlight differential effects of fibrosis on lymphatic vascular growth compared to function. Edemas tend to be associated with impaired drainage and overall loss of lymphatic vascular function,[122–125] while fibroproliferative diseases (e.g., pulmonary fibrosis, renal fibrosis, etc.) often experience excess growth and vascular remodeling.[4,10,82,125] Many tumors, as described previously, perpetuate a fibrotic microenvironment with biophysical and biochemical changes that not only promote capillary growth, but also lower barrier function to allow cells to cross lymphatic capillary walls.[126–128] A subset of these diseases and conditions will be discussed in more detail in subsequent sections to further emphasize the importance of understanding changes to lymphatic vascular growth and function as integrated and separate entities within the context of fibrosis. Inflammation and immune response also play important roles in regulating lymphatic growth and function and will also be discussed in relation to fibrosis and altered tissue microenvironments. Altogether, improved understanding of relationships and interactions unique to fibrosis and lymphatics is integral to informing how we design preclinical models of lymphatic vasculature in disease and develop more effective therapeutic solutions.

6. Lymphangiogenesis in Fibrotic Tumors and Kidney Disease

As previously discussed, lymphangiogenesis is connected to fibrosis, as new lymphatic vessel growth has direct associations with ECM stiffening and soluble factor signaling (e.g., inflammatory) that can have pro- and anti-lymphangiogenic effects.[118] Chronic fibrosis is a risk factor for developing certain cancers, and the links between fibrosis and lymphatic vessel growth have been observed in solid tumors that are characterized by a fibrotic ECM (e.g., breast, pancreatic).[28,30,129,130] and inflammation.[14,131] Within the kidneys, lymphangiogenesis is also closely linked with inflammation and fibrotic progression (Reviewed by Donnan et al., 2021).[132] As such, inflammation within the kidney has a significant negative effect on lymphatic vessel structure and function that exacerbates conditions toward complete renal fibrosis.

In a study of 140 breast cancer patients, Cha et al. correlated lymphangiogenesis with tumor stiffness, measured by shear-wave elastography.[87] Tissue sections of fibrotic tissue areas obtained post-surgery were assessed for lymphatic density and clinically observed disease progression. Together, analyses of tissue stiffness, histological samples, and pathological data revealed that lymphangiogenesis and lymphatic vascular invasion were not only correlated with high tissue stiffness values, but also with the highest histological grade for observing cell morphology toward cancer (Grade 3). High tumor cell proliferation was also observed. These findings support the assertion that fibrosis is a driving factor in lymphangiogenesis in breast cancer, which in turn aids tumor progression.[87] Improved pancreatic tissue clearing and 3D imaging approaches also showed that lymphatic vascular networks are closely associated with pancreatic intraepithelial neoplasia lesions—precursors to highly fibrotic and invasive pancreatic ductal adenocarcinoma—in human and mouse samples.[130] Once pancreatic ductal adenocarcinoma progressed, researchers also observed intra- and peritumoral lymphangiogenesis, indicating that lymphatic capillary growth and remodeling were closely associated with tumor progression. This type of growth and remodeling is regulated, in part, by signaling within the solid tumor

Table 1. Diseases and conditions associated with fibrosis and dysregulated lymphatic vasculature.

Disease or condition	Type of dysregulation	Refs.
Lymphedema and myocardial edema	• Abundant nonfunctional lymphatic capillaries unable to drain interstitial fluid • Accumulated fluid further progresses tissue fibrosis, resulting in further loss of lymphatic capillary function	[122–125]
Fibroproliferative disorders (renal, pulmonary, liver, dermal)	• Excess lymphatic growth in response to fibrosis can cause organ function disruption (e.g., kidney ultrafiltration) • Aberrant mural cell recruitment causes vascular remodeling of the lymphatic tissue • Lymphatic vasculature recruits immune cells, aggravating the inflammatory response and further progressing fibrosis	[4,10,82,125]
Carcinoma	• Tumors use inflammatory and pro-lymphangiogenic cytokines to maintain a fibrotic environment • Abundant vasculature with low barrier function allows for metastasis of the carcinoma into the lymphatic system	[126–128]

microenvironment that sustains inflammatory and fibrotic conditions to put the tumor in a "non-healing wound" state in many cancers.[14,131] For example, continuously activated cancer-associated fibroblasts produce high amounts of VEGF-C to induce lymphangiogenesis and deposit increased amounts of ECM to sustain fibrosis. This process establishes a hypoxia gradient in multiple cancers[87,94] that induces lymphangiogenesis via interactions between HIF-1α, and VEGF isoforms, TGF-β, and Prox1.[94] Moreover, pro-fibrotic M2 polarized macrophages secrete TGF-β, PDGF, and VEGF that can activate fibroblasts and LECs.[133] Within fibrotic tumor microenvironments, tumor-associated macrophages with pro-fibrotic and anti-inflammatory M2 phenotype have been associated with poor prognosis and higher incidence of lymph node metastasis in lung, pancreatic, and colorectal cancers.[134–137] In addition, higher density of peritumoral lymphatic capillaries was also associated with higher numbers of M2 macrophages compared to M1 (pro-inflammatory) macrophages.

Fibrosis-related lymphatic vascular growth is also observed in chronic kidney fibrosis where inflammatory cells play a key role.[29,118] Inflammatory cells, specifically CCR7$^+$ dendritic cells, are recruited during lymphangiogenesis to infiltrate surrounding fibrotic tissue, thus accelerating the inflammatory response and subsequent fibrotic progression. Pei et al. correlated lymphatic vascular growth with the recruitment of CCR7$^+$ dendritic cells by inhibiting lymphangiogenesis with soluble VEGFR3 and LYVE-1 that competitively attached to VEGF-C and HA, respectively. This inhibition weakened the CCR7$^+$ dendritic cell response and attenuated chronic kidney fibrosis progression.[29] As previously discussed, TGF-β plays an important role in fibrosis by supporting increased fibroblast-mediated ECM deposition and promoting lymphangiogenesis via increased VEGF-C production. In fact, TGF-β in the kidney not only induces VEGF-C production in proximal tubule cells and collecting tubule cells, but it also stimulates macrophages and peritoneal mesothelial cells to produce VEGF-C.[111–113] However, the relationship between TGF-β and VEGF-C is complex, as TGF-β also directly inhibits LEC proliferation and migration by downregulating important lymphangiogenic markers (i.e., Prox1, LYVE-1).[110] Macrophages within the kidneys also produce TGF-β and VEGF-C/D and play a significant role in promoting lymphangiogenesis during inflammation and fibrosis. However, their activity differs from tumor-associated macrophages in that the M1 phenotype is more dominant in kidney fibrosis. M1 macrophages have a unique ability to transdifferentiate into LECs through VEGFR3 activation,[138] and a similar phenomenon of transdifferentiation has also been observed in the cornea with CD11b$^+$ macrophages under inflammatory conditions.[15] Transdifferentiation of kidney macrophages was observed through in vitro and in vivo studies.[138] In vitro results showed that M1 macrophages were more likely to form tube-like structures in Matrigel compared to M2 macrophages. Moreover, when M1 and M2 macrophages were injected into mice with fibrotic kidneys, M1 macrophages formed cell clusters or small vessel-like structures within the kidneys, while M2 macrophages were dispersed throughout. The same body of work also showed that VEGF-C exposure induced M0 macrophages to differentiate into M1 macrophages that expressed lymphatic markers before further differentiating into LECs.[138]

Overall, these findings have implications for treatment strategies for tumors and chronic kidney fibrosis as they relate to lymphangiogenesis. Beyond more traditional approaches for targeting lymphangiogenesis directly via anti-growth factor therapies, targeting macrophages or other immune cells (e.g., dendritic cells) may also be an effective approach to interrupt or reverse the effects of fibrosis-associated lymphangiogenesis that sustains these and other similar disease states.

7. Disrupted Lymphatic Function in Chronic Fibrosis and Edema

Chronic fibrosis arises when fibrosis progresses to the point of organ malfunction due to excess scarring, inflammation, reduced blood flow, and impaired oxygen delivery.[8,9] Secondary lymphedema is one example of chronic fibrosis contributing to decreased numbers of functional lymphatic vessels and low overall vascular function. The lack of functioning lymphatic vasculature causes interstitial fluid stasis within affected limbs that lead to acute and chronic edema.[22] Fluid stasis also occurs in myocardial edema in situations when inflammation following myocardial infarction leads to fibrosis and lymphatic vascular dysfunction. The inability to drain interstitial fluid perpetuates fibrosis, which further increases inflammation, alters cardiac ECM mechanics and stiffness, and leads to cardiac failure.[122] Lymphatic vascular dysfunction has been tied to myocardial fibrosis in mouse myocardial infarction models[122,124] and is also linked to the delayed resolution of fibrotic tissue.[25]

Disrupted barrier function observed in edema can lead to a cycle of inflammatory and fibrotic responses that further disrupts lymphatic vascular function.[93] Using a mouse tail lymphedema model, Avraham et al. demonstrated that initial acute edema and subsequent fibrotic progression and inflammation activate and are exacerbated by CD4$^+$ T-cells and T-helper 2 (TH2) cell differentiation.[91] When TH2 cell differentiation was inhibited, fibrotic progression decreased, and lymphatic barrier function improved (increased fluid uptake and drainage). In the same mouse study, bleomycin-induced fibrosis independently inhibited lymphatic vascular function (decreased uptake). The study attributed the development of lymphedema to the initial disruption of lymphatic function that continued to decline with increased subcutaneous tissue fibrosis.[31] In myocardial edema, rapid lymphangiogenesis occurs after injury and fibrosis, primarily due to increased VEGF-C/D expression.[25,46,139] Even though vessel growth increases, the vessels are extremely permeable (low barrier function) to the point where interstitial fluid cannot generate a sufficient pressure gradient to open the capillary lumens. Fluid stasis in damaged myocardial tissue is likely due to low-functioning pre-collecting vessels,[140] decreased fluid propulsion from reduced cardiac contractility,[141,142] and/or initial lymphatic capillary damage.[25,141] One study noted high lymphatic capillary density in injured myocardial tissue, but fewer LYVE-1$^+$/podoplanin$^+$ lymphatic collecting vessels in the fibrotic areas of cardiac tissue.[25] They also attributed myocardial edema, in part, to pre-collecting vessel remodeling in the fibrotic and non-fibrotic portions of the heart that yielded smaller vessels and LYVE-1$^+$/podoplanin$^-$ larger sac-like vessels. Reduced propulsion of lymph fluid through the pre-collecting

vessels also impacts function. One study used lipopolysaccharide and inflammatory cytokines to elicit an acute inflammatory response in mice and noted that lymphatic vascular pumping and propulsion decreased with pro-inflammatory signaling.[143] Collectively, these study outcomes demonstrate a larger role for lymphatic vascular function compared to growth in edemas. However, the question remains for how lymphatic vessel function can be restored.

Although VEGF-C is a standard pro-lymphangiogenic treatment to stimulate vessel growth, lymphatic function is not always restored with VEGF-C stimulation.[25] Instead, treatment with VEGF-C or other pro-lymphangiogenic factors may actually increase the growth of dysfunctional vessels in situations where dysfunction is the distinctive pathological feature. Alternatively, additional signaling factors may alter or hinder pro-lymphangiogenic effects.[90–92] For example, Avraham et al. showed that blocking TGF-β1 signaling in a mouse tail lymphedema model reduced fibrosis, lowered inflammation, improved lymphatic function, and increased lymphangiogenesis.[23,93] Ogino et al. observed contrasting results when they transplanted adipose-derived stem cells into a hind leg lymphedema mouse model. Increased TGF-β1 production stimulated LEC proliferation, increased the number of lymphatic vessels, and reduced the severity of the disease.[125] In both cases, the disease state improved evidenced by reduced fibrosis severity, reduced lower limb volume, and some degree of lymphatic growth, yet the intervention strategy differed (inhibition vs stimulation). These differences could be attributed to the type of lymphedema model—tail[23,93] versus hind limb[125], but there were also differences in the timing and duration of intervention. In the studies from Avraham et al., the TGF-β1 blockade was introduced 24 h before the injury (surgery or irradiation) occurred.[23,93] Since TGF-β1 signaling is typically upregulated in fibrosis, applying the treatment before injury may have had more of a prophylactic effect against the onset of fibrosis and related inflammatory response in the tail model, evidenced by decreased expression of inflammatory cytokines—IL-4 and IL-13—in one of the studies.[93] On the other hand, Ogino et al. initiated TGF-β1 production via adipose-derived stem cells 24 h after irradiating the hind limb.[125] Since tissues exhibited swelling within 24 h of injury, treatment started after fibrosis. However, even though tissues without adipose-derived stem cells showed evidence of fibrosis via type I collagen production, there was no increase in TGF-β1 mRNA expression over time. This result was unexpected given that TGF-β1 is typically upregulated in fibrosis, but since adipose-derived stem cells secrete factors that suppress inflammation, they may have inhibited TGF-β1 production from resident immune cell populations. Adipose-derived stem cells also secrete many pro-lymphangiogenic factors such as FGF and hepatocyte growth factor (HGF), which likely explains why functional lymphatic vessels still grew even though VEGF-C expression was suppressed in groups with adipose-derived stem cells. Overall, these results highlight some of the nuances of lymphangiogenesis and related signaling, particularly the dual nature of TGF-β activity in lymphangiogenesis. Moreover, study outcomes highlight the complex connection between fibrosis and lymphatic growth and function and the potential importance of timing in considering therapeutic intervention.

8. Growth versus Function in Early and Late-Stage Disease

As fibrosis progresses toward a long-term chronic stage, the effects of the altered ECM and biochemical microenvironment impact lymphatic vascular growth and maintenance over time. It is important to understand the temporal nature of lymphatic vascular growth and function during fibrotic progression because the corresponding pathophysiological changes can dictate the choice of treatment and corresponding treatment efficacy. Furthermore, as we identify specific pathological features that separately relate to growth and function to predict disease progression and severity, the following question arises: *"Can a disease hallmarked by lymphatic vascular dysfunction be predicted by rapid and excessive lymphatic vessel growth?"*

Ogata et al. found that excessive lymphangiogenesis occurred during the early stages of induced lymphedema in a mouse abdominal model.[83] Researchers visualized new vascular growth and excess LECs in the tissues surrounding the existing collecting lymphatic vasculature, suggesting that LEC invasion into tissues is a precursor for initiating lymphangiogenesis. A similar result was observed in human lymphedematous tissue that also showed robust networks of lymphatic capillaries surrounding the collecting lymphatic vasculature.[31] Overall increases in early lymphangiogenesis in these studies were tied to $CD4^+$ T cell, T-helper 1 cell (TH1), and T-helper 17 cell (TH17) activation, and lymphangiogenesis decreased when those T cell populations were suppressed. Furthermore, Ogata et al. found that the rapid growth of new lymphatic vasculature was predictive of lymphedema development, and suppressing T-helper cell activation decreased both lymphedema and fibrotic progression. Their observations[21] aligned with results from a previously described study by Avraham et al.,[31] but the two studies presented different explanations for their observations and focused on different outcomes (growth vs function).[83,91] While the main conclusion from Avraham et al. was that suppression of $CD4^+$ cells from early onset lymphangiogenesis and subsequent suppression of the fibrotic and inflammatory responses were primarily responsible for restoring lymphatic function,[31] Ogata et al. noted that $CD4^+$ cells interacted with lesional macrophages to promote lymphangiogenesis.[21] Furthermore, suppressing VEGF-C, which usually reduces vascular growth, significantly reduced edematous tissue in the Ogata study. This result suggests that the pathological lymphangiogenesis they observed was responsible, in part, for lymphatic dysfunction (poor drainage) despite vascular growth.[83]

Researchers must remember that growth does not equal function and that more lymphatic vascular growth is not always advantageous, especially if the newly formed vessels are not functional. Moreover, resolving vascular insufficiency by targeting either growth or function may be dependent on the stage of disease progression. A key component of the findings from Ogata et al. was the high permeability of newly formed vasculature.[21] As early as the second day of their study, excessive leakage of Evans blue dye from lymphatic vessels was observed, especially around the newly formed vessels. Suppressing VEGF-C reduced dye leakage and significantly suppressed fibrotic development within the tissue. The authors also acknowledged previous work in which

promoting lymphangiogenesis was therapeutically advantageous for treating secondary lymphedema and other inflammatory pathologies, specifically by resolving acute inflammation earlier in the process prior to restoringlymphatic vascular function.[144,145] They also hypothesized that the involvement of TH1 and TH17 cells may be responsible for the acute inflammatory and early lymphangiogenic responses, whereas TH2 cells may be responsible for suppressing lymphatic vessel function and maturation in later stages.[83] However, questions remain concerning the proposed T-helper cell involvement, and there has been limited follow-up on this work, to our knowledge. Also, Ogata et al. made a point to distinguish lymphangiogenesis from lymphatic vascular function, which highlights the importance of using specific language when discussing lymphangiogenesis and lymphatic vascular function. Consistent, accurate, and intentional terminology will allow for more nuanced discussions around the transition from early to late-stage fibrotic disease states and how researchers can effectively target growth and function for improved therapeutic efficacy.

9. Tissue Engineered Models of Lymphatic Vasculature

Most of the studies discussed up to this point were performed in rodent models that allow researchers to assess the systemic response to changes in fibrosis, lymphangiogenesis, lymphatic vascular function, or immune cell response.[21,23,31,93] Animal models can also be used to study the stages of lymphatic vessel growth and related vessel function during disease initiation and progression. However, there is a need to establish similarly descriptive in vitro humanized models of fibrosis in varied disease states in which lymphangiogenesis and lymphatic vascular function can be studied systematically. Beyond gaining fundamental knowledge of relevant mechanisms that drive lymphatic vascular growth and function under fibrotic conditions, specific details of progressive fibrotic diseases and conditions need to be clarified to properly derive therapeutic strategies toward curative solutions. Researchers are using tissue engineering and microfabrication approaches (**Figure 3**) to model key features of lymphatic vasculature and the surrounding microenvironment in vitro to study specific mechanisms that regulate lymphangiogenesis, recapitulate certain disease states with lymphatic insufficiencies, and screen potential therapeutic strategies. Although tissue engineering is traditionally associated with tissue or organ regeneration, it lends itself well to tissue modeling as a means for studying tissue function and disease. Others have recently provided reviews on lymphatic tissue engineering as it relates to modeling and lymphatic regeneration (Reviewed[146,147]). For the current review, we have chosen to provide a general overview of lymphatic tissue engineering strategies before focusing on approaches that are more aligned with modeling lymphatic growth and function alongside features of fibrosis (e.g., microfluidics, ECM stiffness).

Tissue-engineered in vitro models have the advantage of being able to integrate biophysical, biomechanical, and/or biochemical cues for independent systematic study that is more robust than conventional 2D in vitro models on tissue culture plastic (Figure 3A) that do not have any tissue- or disease-specific features beyond soluble factor signaling. One common tissue engineering-based approach involves 3D hydrogels that can support LEC spheroids (Figure 3B) to monitor lymphatic sprouting over time as an indicator of lymphangiogenic activity.[148–150] Alternatively, cells—LECs and stromal cells—can be encapsulated and distributed throughout 3D hydrogels, which may result in LECs forming tube-like or capillary-like structures. Encapsulated cells can also be evaluated on their migratory behavior through a 3D hydrogel that mimics an interstitial ECM or assessed for phenotypic changes in response to altered ECM properties. When LECs are cultured on top of a 3D hydrogel, they can still sense ECM properties and often have similar measured outcomes as seen in encapsulated samples.[104,147,151] One limitation of the 3D hydrogel-based model for lymphatic vasculature is that it is a static model that lacks dynamic elements observed in lymphatic vasculature in vivo, namely biomechanical inputs such as fluid shear, pressure, and stretch. To address this limitation, researchers use microfluidic devices (Figure 3C) of varied designs with multiple channels, compartments, inlets, and outlets to not only apply various biomechanical inputs to the system, but also establish chemokine/soluble factor gradients to promote lymphatic sprouting from LEC-lined channels into an interstitial matrix (Figure 3D,E).

9.1. Microfluidic-Based Approaches for Modeling Lymphatic Vasculature

Microfluidic chip technologies that support 3D hydrogel cultures have been developed extensively for studying angiogenesis for blood vessel research (Reviewed[152–154]). More recently, they have been adapted for studying lymphangiogenesis with passive or active fluid flow to observe the LEC response to fluid shear stress and subsequent migration and lymphangiogenic sprouting into the hydrogel.[116,152,155] To recapitulate lymphangiogenic growth from an existing lymphatic vessel into a surrounding ECM, microfluidic devices can be designed with two channels separated by an ECM barrier. One channel is seeded with LECs, and the second channel is used to establish a chemokine gradient that diffuses across the ECM (i.e., hydrogel) to drive lymphangiogenic sprouting into the ECM. The chemokine gradient is particularly important because soluble factors help drive proliferation, migration, and eventual lymphangiogenesis. Potent pro-lymphangiogenic growth factors such as VEGF-C, basic FGF, and HGF are well established; however, unknown soluble factors produced by activated fibroblasts or other stromal cell populations also drive lymphangiogenesis. For example, conditioned medium from adipose-derived stem cells induced lymphatic vascular growth in a microfluidic model of lymphedema.[156] The concentrations of lymphangiogenic growth factors within the conditioned media were much lower than the concentrations of recombinant proteins used to produce similar amounts of LEC proliferation, migration, tube formation, and sprouting, which highlighted the role of stromal adipose tissue surrounding lymphatic vessels in regulating lymphatic vascular growth. Nonetheless, vessel function was not evaluated.

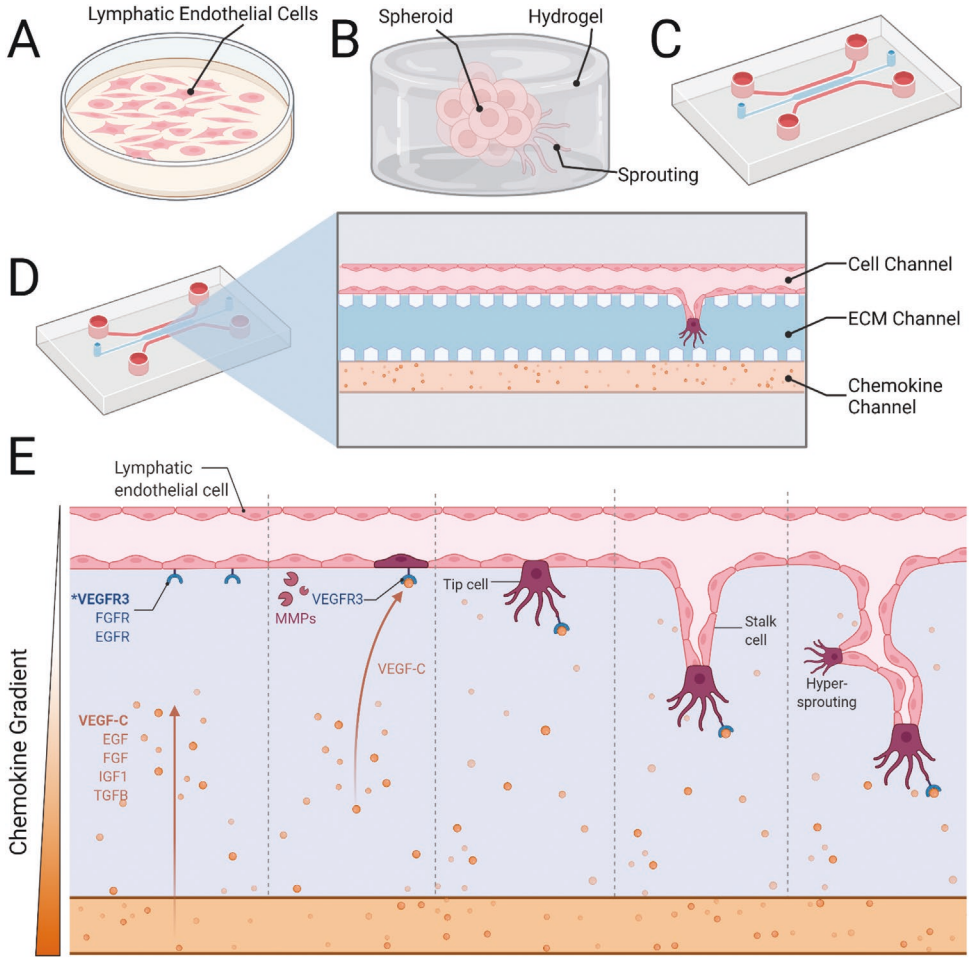

Figure 3. Increasing complexity of in vitro models of lymphatic vascular growth is shown. A) 2D LEC growth on tissue culture plastic or similar substrates use proliferation as an indicator of vascular growth. B) 3D spheroid culture, utilizes both proliferation and vascular sprouting as indicators of vascular growth. C,D) 3D microfluidic chip models, allows for measurement of proliferation, sprouting morphogenesis, barrier function, and vascular perfusion and persistence. E) Detailed illustration of the progression of vascular sprouting from a central lymphatic vessel within a microfluidic device with established chemokine gradients. Created with BioRender.com.

Interstitial flow is an important biomechanical cue related to lymph fluid flow, and it changes over time as fibrosis progresses due to changes in ECM biophysical properties (e.g., fiber density, porosity).[157] With fibrosis, interstitial fluid pressure increases and flows away from the increasingly stiff and protein-dense microenvironment. Microfluidic devices have been adapted to include interstitial flow parallel to the growth direction of the lymphatic vasculature by establishing a pressure differential. The mechanical stress imparted by this interstitial fluid flow helps regulate lymphangiogenic processes. A study by Kim et al. showed that there is a pro-lymphangiogenic response when the interstitial flow is against the direction of vascular growth and suppressed when flow is in the direction of growth.[157] However, since those vessels grew within a soft fibrin gel (≈1 kPa), the microenvironment was not reflective of ECM stiffness levels observed within a fibrotic microenvironment. This stiffness gap limits the model's utility to function as a fibrotic disease model and lacks any dynamic fibrotic elements beyond altered interstitial flow patterns.

9.2. ECM Stiffness and Lymphatic Vasculature

Over the last few years, there has been increasing interest in investigating the role of ECM stiffness in driving certain lymphatic vessel behaviors. Material-based tissue engineering approaches that use biomaterials at varied ECM stiffness values have provided opportunities to study the direct impact of stiffness on LECs and lymphatic vessels. However, a search for research articles that directly address ECM stiffness and lymphatic vasculature only returns a handful of search results. Some recent studies have shown that ECM stiffness drives specific steps in early lymphangiogenesis that are primarily associated with fetal development. Frye et al. cultured human dermal LECs on fibronectin-coated dishes to create soft substrates of similar stiffness to early chicken embryos or adult brain tissue (0.2 kPa).[104] Stiffer substrates mimicked the stiffness of the cardinal vein (4 kPa), the source of lymphatic endothelial progenitor cells during development, and the stiffest substrates were more representative of muscle (8 and 12 kPa) and bone (25 kPa). The study noted over 2500 transcriptional changes in

LECs on soft substrates compared to stiff substrates and found that cell migration, cell–matrix adhesion, and vascular development-related genes were upregulated on soft substrates, while cell proliferation-related genes were downregulated. GATA2 transcription, which is upregulated in response to oscillatory flow and helps regulate lymphatic valve formation[106] was upregulated in LECs cultured on soft substrates (0.2–0.3 kPa). Genes for matrix metalloproteinases 1, 2, and 10, which are positive regulators of lymphangiogenesis, were also upregulated on soft substrates, in addition to GATA2-dependent genes associated with locomotion, motility, and migration.[104]

More recently, Alderfer et al. used hydrogels comprised of thiol-modified HA conjugated with heparin, thiol-modified gelatin, and varied ratios of polyethylene glycol diacrylate to generate soft (30 Pa), medium (300 Pa), and firm (900 Pa) substrates for LECs.[107] They demonstrated how LECs cultured on softer matrices readily formed cord-like structures when exposed to high VEGF-C concentrations (50 ng mL^{-1}) compared to stiffer matrices. Their approach also allowed them to see that matrix stiffness appeared to be a major factor directing VEGFR3 activation independent of VEGF-C concentration. Both activation of VEGFR3 and expression of Prox1 were enhanced on softer matrices, as were genes for matrix metalloproteinases 2 and 14 that are involved in cell migration and tube formation. Altogether, these molecules, along with those identified in Frye et al., are involved in multiple elements of lymphangiogenesis, including cell migration, lymphatic sprouting, and vessel growth.[104] Moreover, the observed decrease in lymphangiogenic behaviors in LECs cultured on stiffer substrates and overall preference for softer substrates in both studies may provide some evidence to support that ECM stiffness increases caused by fibrosis are inhibitory to lymphatic vascular function.

By altering stiffness independent of substrate composition, researchers tuned substrate stiffness while maintaining ligand density. By limiting the influence of confounding factors, researchers like Frye and Alderfer have started to establish links between ECM stiffness, mechanosensing molecules, and capillary tube formation.[102,107] However, the studies were more focused on developmental lymphangiogenesis rather than late-stage lymphangiogenesis that occurs in injury and disease. The hydrogel formulation used by Alderfer et al. achieved stiffness values similar to tissues surrounding the cardinal veins during embryonic development,[107] while Frye et al. used a wide range of stiffness values that also included stiffer mature tissues.[104] Despite having the ability to achieve higher stiffness values, the latter study's primary goal was still to study developmental lymphangiogenesis. Thus, there is an opportunity to use these and other tissue engineering approaches to broaden this area of research beyond these few stiffness studies.

10. Future Directions and Opportunities

Overall, this review of lymphatic vascular biology within the context of fibrosis highlights important relationships between fibrotic microenvironments, lymphangiogenesis, and lymphatic function. Yet, gaps in knowledge surrounding fibrosis and lymphatics remain, particularly when considering how dependent lymphatic vascular outcomes are on the dynamic biochemical, biomechanical, and biophysical microenvironments that occur with fibrosis. Gaps in fundamental understanding also persist when experimental models lack the full capacity to systematically study temporal changes in microenvironmental features and approximate fibrotic disease states. In vitro modeling and tissue engineering offer opportunities to study key interactions in a more systematic way compared to animal models, and progress has been made to design and develop more complex and pathophysiologically relevant in vitro models of lymphatic vasculature. However, the field still lacks comprehensive modeling of lymphatic vascular growth and function within fibrotic disease environments that combine microenvironmental features that reflect multiple elements of fibrosis, including ECM stiffness and key temporal changes in microenvironmental features. Also, even though there are some tissue-engineered in vitro models of lymphangiogenesis that are designed to reflect aspects of different fibrotic diseases,[114,156–159] to our knowledge there is no model that has been predictive of what is observed in vivo. This mismatch of outcomes is directly related to limited therapeutic strategies that are available to patients beyond symptom management. Patients need curative options, and although it may seem challenging, the fields of lymphatic vascular biology and tissue engineering have numerous opportunities to be leaders in enhancing in vitro model development and improving restorative strategies for lymphatic vasculature. Although tissue engineering strategies can also be used for regeneration to restore lymph nodes and lymphatic vessels, that research is beyond the scope of this review article and has been reviewed by others.[146,160]

From the tissue engineering perspective, this review primarily focuses on how researchers use hydrogel- and microfluidic-based in vitro models to gain insights into biochemical, biomechanical, and biophysical factors that influence lymphangiogenesis.[116,156,157] Lymphatic research has traditionally focused on the impacts of fluid flow, cyclic stretch, and interstitial pressure on lymphatic vascular outcomes.[5,161,162] However, increased emphasis has been placed on studying biochemical signaling that arises from physical and paracrine interactions with stromal cell populations with and without interstitial flow. One such study found that renal tubular activated fibroblasts, which are present in kidney disease, produced sonic hedgehog (soluble factor) that increased proliferation in LECs but not blood endothelial cells in vitro and in vivo (animal model).[159] Similarly, adipose-derived stem cells also promote vascular growth in lymphedema and dermal fibrosis models,[125,156] and other studies have identified roles for T-helper cells.[21,31] These results support evidence that activated stromal cells in fibrotic diseases help promote lymphatic vessel growth. However, broad studies across diseases are limited, and much of the work has been performed in vivo animal models rather than in humanized in vitro models. Expanded studies and in vitro models that incorporate disease-specific stromal populations are needed, especially those that investigate the roles of immune cells beyond T-helper cells, such as macrophages.[138] Moreover, most studies do not consider stiffness as an experimental variable to explicitly investigate the impact of in vivo tissue stiffness changes, and the in vitro models do not use pathophysiological stiffness values, despite evidence that stiffness is an important mechanical cue and regulator of lymphatic growth.[30,104,107]

More recent studies that focus on the role of ECM stiffness in directing lymphangiogenesis under developmental conditions represent a new generation of lymphatic vascular studies that significantly benefit from tissue engineering approaches and perspectives.[104,107] Yet, these stiffness studies are few, and like many others, stop short of fully recapitulating fibrotic tissue characteristics in vitro. Studies often use Matrigel, which is softer than many healthy tissues, or they use tissue culture plastic, which is stiffer than most diseased tissues. However, there are numerous natural and synthetic biomaterials that can be used to modulate the lymphatic system in vitro (Reviewed by Alderfer et al. 2021).[138] The stiffness of many of those biomaterials can be tuned to have stiffness values that are more aligned with healthy tissues in development,[104,107] while other materials may need to undergo certain modifications to achieve higher stiffness values that represent diseased tissues experiencing fibrosis. Moreover, in vitro model designs that include ECM stiffness as a key variable present LECs with static ECM stiffness that does not mimic the progressive nature of ECM stiffening over time during fibrosis. We hypothesize that these oversights may contribute, in part, to the observed mismatch between in vitro results and in vivo outcomes, particularly since the temporal nature of fibrosis and related inflammation have been implicated in the timing and success of treatments of lymphatic insufficiencies. Therefore, there is a need for models that include a wider range of dynamic components to better recapitulate the progressive nature of fibrosis, which includes biophysical and biochemical changes over time. A dynamic model could include a matrix component with temporal stiffening capabilities or microfluidic chips that support interstitial flow and soluble factor gradients through a 3D ECM. These designs can be adapted to examine questions specific to lymphangiogenesis, such as how progressive stiffening impacts LEC response to well-characterized chemokines (e.g., VEGF-C, TGF-β) and chemokines produced by stromal and tumor cell populations, as well as subsequent vascular growth/sprouting and vessel maturation.

There are additional opportunities to design models or develop strategies that enable straightforward measurements of vessel barrier function and perfusability independent of lymphatic vessel growth. The ability to study lymphatic vessel function and fibrosis in the same model is of high importance since the biochemical and biophysical environment of fibrotic tissues plays a significant role in regulating vessel function. Although transwell inserts with and without an ECM layer have been used to study transport across 2D monolayers of LECs,[101,163] the format cannot account for transport across the walls of intact vasculature within a 3D matrix. To move the field forward, lymphatic biology has taken a cue from blood vessel studies where barrier function has been examined using microfluidic chips with a hydrogel.[115,164] In one study, a single microchannel was created within a type I collagen hydrogel on a microfluidic chip, and human dermal LECs lined the microchannel to generate a central or primary lymphatic vessel. Fluorescently labeled dextran was added to the cell culture medium, and changes in fluorescence intensity across the central vessel wall were monitored to calculate lymphatic permeability.[115] The researchers also added fibronectin to the collagen to study the impact of ECM composition on vessel permeability and determined that the presence of fibronectin and/or α5 integrin activation tightened LEC junctions and lowered vessel permeability. Although this approach shares some similarities with more traditional transwell migration studies, the presence of microchannels within a 3D hydrogel expands the research questions that can be asked with microfluidic chips, particularly those that involve more dynamic elements such as laminar flow (active) and interstitial flow (active or passive). Hydrogels can also be seeded with stromal cells, disease-specific cells, or macromolecules to study the impact of cell–ECM and cell–cell interactions (direct or indirect) on lymphatic vessel growth and function. Moreover, as microfluidic chip designs are updated to include additional channels that support chemokine gradients or additional central LEC vessels, capillary-like vessel sprouting will occur, much like what is observed in blood vessel-on-a-chip studies. Therefore, it will also be important to extend function testing to those sprouting lymphatic vessels as small molecules flow through the primary vessels (i.e., LEC-lined microchannels). Overall, functional assessment of barrier function and perfusability is still a newer area of study for lymphatic studies compared to blood vessels,[164] especially when we consider that barrier function is rarely addressed in the lymphatic vessels that sprout from a primary vessel into a surrounding ECM. The gaps widen even more when wanting to study the impact of fibrosis and fibrotic progression on lymphatic capillary function. Without fully considering barrier function alongside lymphangiogenesis when studying fibrosis and disease, the field puts itself at a significant disadvantage in the pursuit of new and effective treatment strategies for patients.

11. Concluding Remarks

Overall, this review has some key takeaways related to the biological understanding of lymphatic vasculature under fibrotic conditions:

First, fibrosis is a progressive process that occurs in numerous conditions and diseases that also involve lymphatics, and depending on the pathological state, fibrosis can enhance or inhibit lymphatic vascular growth and function. Current therapeutic strategies for targeting fibrosis or directly targeting lymphatic vasculature have limited efficacy, due in large part to incomplete understanding of the biology of fibrosis-mediated responses observed in lymphatics. Therefore, we must be more intentional and utilize dynamic models that can replicate progressive changes in the tissue microenvironment over time to better inform the development of targeted therapeutics.

Second, the distinction between lymphangiogenesis and lymphatic vascular function as measures of lymphatic vascular health also needs to be specified and better understood in the context of microenvironmental changes within the surrounding tissue. In general, biochemical cues related to fibrosis appear to induce lymphatic vascular growth, while biophysical cues observed in fibrosis (i.e., stiffness) appear to negatively impact lymphatic vascular function.[12,21,23,24,31,93,107] The combination of increased growth and decreased function can often result in more traditional pathological lymphangiogenesis with leaky

vasculature and subsequent fibrosis creating a positive feedback loop to perpetuate the pathology.[13,28]

Finally, the study of lymphatic tissue engineering is a budding scientific field, as more research reveals that the lymphatic system is not just a passive highway for the immune system and improved understanding of lymphatic vascular biology is needed. Animal models have been successful in studying systemic body responses to fibrosis-induced lymphangiogenesis and lymphatic vessel dysfunction, but there is a place for in vitro models when studying individual contributions of specific variables and systematic interactions between variables. Current in vitro models of lymphatic vasculature, specifically models that are applied toward lymphatics in fibrotic disease, do not often include both growth and function as measured outcomes. Most current models focus solely on lymphatic vascular growth, or even just LEC proliferation and migration as proxies for vascular growth. Although these models produce useful information and have helped advance the field, they still do not tell the complete story. Furthermore, elements of fibrosis, such as critical biophysical cues (e.g., ECM stiffness) and the temporal change in biophysical and biochemical signaling, have largely been left out of these models with a few exceptions.

Overall, while lymphatic research has seen a resurgence in recent years, there is still much work to be done. We believe that fibrosis and lymphangiogenesis research needs to continue to adopt more comprehensive perspectives on lymphatic vascular biology to appropriately address knowledge gaps surrounding fibrosis-related lymphatic capillary growth and function in disease progression. As researchers continue to increase the complexity of preclinical models, particularly in vitro systems, they will be better equipped to fully investigate the lymphatic system. Moreover, an improved understanding of lymphatic vascular biology resulting from better model designs will help resolve the discrepancies in outcomes between in vitro and in vivo models and advance the development of more effective therapeutic options for patients with lymphatic insufficiencies.

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Conflict of Interest

The authors declare no conflict of interest.

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[1] C. Norrmén, T. Tammela, T. V. Petrova, K. Alitalo, *Circulation* **2011**, *123*, 1335.
[2] F. Zhang, G. Zarkada, S. Yi, A. Eichmann, *Front. Physiol.* **2020**, *11*, 509.
[3] B. W. Wong, A. Zecchin, M. García-Caballero, P. Carmeliet, *Dev. Cell* **2018**, *45*, 289.
[4] T. V. Petrova, G. Y. Koh, *J. Exp. Med.* **2018**, *215*, 35.
[5] A. Mukherjee, J. B. Dixon, in *Vascular Mechanobiology in Physiology and Disease* (Eds: M. Hecker, D. J. Duncker), Springer International Publishing, New York **2021**, pp. 191–239.
[6] T. Tammela, K. Alitalo, *Cell* **2010**, *140*, 460.
[7] H. Kinashi, Y. Ito, T. Sun, T. Katsuno, Y. Takei, *Int. J. Mol. Sci.* **2018**, *19*, 2487.
[8] T. Wynn, *J. Pathol.* **2008**, *214*, 199.
[9] J.-I. Jun, L. F. Lau, *J. Clin. Invest.* **2018**, *128*, 97.
[10] J. Liu, C. Yu, *Int. J. Mol. Sci.* **2022**, *23*, 6970.
[11] J. Majo, B. M. Klinkhammer, P. Boor, D. Tiniakos, *Curr. Opin. Pharmacol.* **2019**, *49*, 82.
[12] T. Avraham, N. W. Clavin, S. V. Daluvoy, J. Fernandez, M. A. Soares, A. P. Cordeiro, B. J. Mehrara, *Plast. Reconstr. Surg.* **2009**, *124*, 438.
[13] G. Pei, Y. Yao, Q. Yang, M. Wang, Y. Wang, J. Wu, P. Wang, Y. Li, F. Zhu, J. Yang, Y. Zhang, W. Yang, X. Deng, Z. Zhao, H. Zhu, S. Ge, M. Han, R. Zeng, G. Xu, *Sci. Adv.* **2019**, *5*, eaaw5075.
[14] H. F. Dvorak, *Cancer Immunol. Res.* **2015**, *3*, 1.
[15] K. Maruyama, M. Ii, C. Cursiefen, D. G. Jackson, H. Keino, M. Tomita, N. Van Rooijen, H. Takenaka, P. A. D'amore, J. Stein-Streilein, D. W. Losordo, J. W Streilein, *J. Clin. Invest.* **2005**, *115*, 2363.
[16] A. W. Lund, T. R. Medler, S. A. Leachman, L. M. Coussens, *Cancer Discovery* **2016**, *6*, 22.
[17] B. M. Stramer, R. Mori, P. Martin, *J. Invest. Dermatol.* **2007**, *127*, 1009.
[18] M. Kawaguchi, M. Takahashi, T. Hata, Y. Kashima, F. Usui, H. Morimoto, A. Izawa, Y. Takahashi, J. Masumoto, J. Koyama, M. Hongo, T. Noda, J. Nakayama, J. Sagara, S. Taniguchi, U. Ikeda, *Circulation* **2011**, *123*, 594.
[19] W. E. Cromer, S. D. Zawieja, B. Tharakan, E. W. Childs, M. K. Newell, D. C. Zawieja, *Angiogenesis* **2014**, *17*, 395.
[20] L.-C. Yao, P. Baluk, R. S. Srinivasan, G. Oliver, D. M. McDonald, *Am. J. Pathol.* **2012**, *180*, 2561.
[21] F. Ogata, K. Fujiu, S. Matsumoto, Y. Nakayama, M. Shibata, Y. Oike, I. Koshima, T. Watabe, R. Nagai, I. Manabe, *J. Invest. Dermatol.* **2016**, *136*, 706.
[22] J. H. Dayan, C. L. Ly, R. P. Kataru, B. J. Mehrara, *Annu. Rev. Med.* **2018**, *69*, 263.
[23] T. Avraham, A. Yan, J. C. Zampell, S. V. Daluvoy, A. Haimovitz-Friedman, A. P. Cordeiro, B. J. Mehrara, *Am. J. Physiol.: Cell Physiol.* **2010**, *299*, C589.
[24] L. Song, X. Chen, T. A. Swanson, B. Laviolette, J. Pang, T. Cunio, M. W. Nagle, S. Asano, K. Hales, A. Shipstone, H. Sobon, S. D. Al-Harthy, Y. Ahn, S. Kreuser, A. Robertson, C. Ritenour, F. Voigt, M. Boucher, F. Sun, W. C. Sessa, R. J. R. Flach, *eLife* **2020**, *9*, e58376.
[25] O. Henri, C. Pouehe, M. Houssari, L. Galas, L. Nicol, F. Edwards-Lévy, J.-P. Henry, A. Dumesnil, I. Boukhalfa, S. Banquet, D. Schapman, C. Thuillez, V. Richard, P. Mulder, E. Brakenhielm, *Circulation* **2016**, *133*, 1484.
[26] A. Alitalo, M. Detmar, *Oncogene* **2012**, *31*, 4499.
[27] J. Stachura, M. Wachowska, W. W. Kilarski, E. Güç, J. Golab, A. Muchowicz, *Oncoimmunology* **2016**, *5*, e1182278.
[28] R. C. Ji, *Cancer Lett.* **2014**, *346*, 6.
[29] G. Pei, Y. Yao, Q. Yang, M. Wang, Y. Wang, J. Wu, P. Wang, Y. Li, F. Zhu, J. Yang, Y. Zhang, W. Yang, X. Deng, Z. Zhao, H. Zhu, S. Ge, M. Han, R. Zeng, G. Xu, *Sci. Adv.* **2019**, *5*, eaaw5075.

[30] Y. J. Cha, J. H. Youk, B. G. Kim, W. H. Jung, N. H. Cho, *Yonsei Med. J.* **2016**, *57*, 599.
[31] T. Avraham, J. C. Zampell, A. Yan, S. Elhadad, E. S. Weitman, S. G. Rockson, J. Bromberg, B. J. Mehrara, *FASEB J.* **2013**, *27*, 1114.
[32] O. Henri, C. Pouehe, M. Houssari, L. Galas, L. Nicol, F. Edwards-Lévy, J.-P. Henry, A. Dumesnil, I. Boukhalfa, S. Banquet, D. Schapman, C. Thuillez, V. Richard, P. Mulder, E. Brakenhielm, *Circulation* **2016**, *133*, 1484.
[33] W. Zheng, A. Aspelund, K. Alitalo, *J. Clin. Invest.* **2014**, *124*, 878.
[34] A. Finnane, M. Janda, S. C. Hayes, *Am. J. Phys. Med. Rehabil.* **2015**, *94*, 483.
[35] Y. Gu, X. Qi, S. Guo, *Clin. Exp. Metastasis* **2008**, *25*, 717.
[36] A. González-Loyola, T. V. Petrova, *Adv. Drug Delivery Rev.* **2021**, *169*, 63.
[37] G. A. Secker, N. L. Harvey, *Int. J. Mol. Sci.* **2021**, *22*, 7760.
[38] M. H. Ulvmar, T. Mäkinen, *Cardiovasc. Res.* **2016**, *111*, 310.
[39] S. Lutter, T. Makinen, in *Developmental Aspects of the Lymphatic Vascular System* (Eds: F. Kiefer, S. Schulte-Merker), Springer, Berlin **2014**, p. 55.
[40] S. Coso, E. Bovay, T. V. Petrova, *Blood* **2014**, *123*, 2614.
[41] D. Choi, E. Park, E. Jung, Y. J. Seong, J. Yoo, E. Lee, M. Hong, S. Lee, H. Ishida, J. Burford, J. Peti-Peterdi, R. H. Adams, S. Srikanth, Y. Gwack, C. S. Chen, H. J. Vogel, C. J. Koh, A. K. Wong, Y.-K. Hong, *J. Clin. Invest.* **2017**, *127*, 1225.
[42] A. Aspelund, M. R. Robciuc, S. Karaman, T. Makinen, K. Alitalo, *Circ. Res.* **2016**, *118*, 515.
[43] J. W. Breslin, Y. Yang, J. P. Scallan, R. S. Sweat, S. P. Adderley, W. L. Murfee, *Compr. Physiol.* **2018**, *9*, 207.
[44] L. Yao, D. M. Mcdonald, in *Developmental Aspects of the Lymphatic Vascular System* (Eds: F. Kiefer, S. Schulte-Merker), Vol. *214*, Springer, Berlin **2014**, p. 41.
[45] I. Martinez-Corral, M. H. Ulvmar, L. Stanczuk, F. Tatin, K. Kizhatil, S. W. M. John, K. Alitalo, S. Ortega, T. Makinen, *Circ. Res.* **2015**, *116*, 1649.
[46] L. Klotz, S. Norman, J. M. Vieira, M. Masters, M. Rohling, K. N. Dubé, S. Bollini, F. Matsuzaki, C. A. Carr, P. R. Riley, *Nature* **2015**, *522*, 62.
[47] L. Stanczuk, I. Martinez-Corral, M. H. Ulvmar, Y. Zhang, B. Laviña, M. Fruttiger, R. H. Adams, D. Saur, C. Betsholtz, S. Ortega, K. Alitalo, M. Graupera, T. Mäkinen, *Cell Rep.* **2015**, *10*, 1708.
[48] N. Escobedo, G. Oliver, *Annu. Rev. Cell Dev. Biol.* **2016**, *32*, 677.
[49] A. Sabine, E. Bovay, C. S. Demir, W. Kimura, M. Jaquet, Y. Agalarov, N. Zangger, J. P. Scallan, W. Graber, E. Gulpinar, B. R. Kwak, T. Mäkinen, I. Martinez-Corral, S. Ortega, M. Delorenzi, F. Kiefer, M. J. Davis, V. Djonov, N. Miura, T. V. Petrova, *J. Clin. Invest.* **2015**, *125*, 3861.
[50] J.-J. Chiu, S. Chien, *Physiol. Rev.* **2011**, *91*, 327.
[51] P. Baluk, J. Fuxe, H. Hashizume, T. Romano, E. Lashnits, S. Butz, D. Vestweber, M. Corada, C. Molendini, E. Dejana, D. M. Mcdonald, *J. Exp. Med.* **2007**, *204*, 2349.
[52] L. K. Chang, G. Garcia-Cardeña, F. Farnebo, M. Fannon, E. J. Chen, C. Butterfield, M. A. Moses, R. C. Mulligan, J. Folkman, A. Kaipainen, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11658.
[53] Y. Cao, *Cell Cycle* **2005**, *4*, 231.
[54] X. Zhang, J. E. Groopman, F. W. Jian, *J. Cell. Physiol.* **2005**, *202*, 205.
[55] L. Planas-Paz, B. Strilić, A. Goedecke, G. Breier, R. Fässler, E. Lammert, *EMBO J.* **2012**, *31*, 788.
[56] K. Vaahtomeri, S. Karaman, T. Mäkinen, K. Alitalo, *Genes Dev.* **2017**, *31*, 1615.
[57] T. V. Petrova, G. Y. Koh, *Science* **2020**, *369*, eaax4063.
[58] S. Eccles, L. Paon, J. Sleeman, *Clin. Exp. Metastasis* **2007**, *24*, 619.
[59] P. Baluk, D. M. McDonald, *Cold Spring Harbor Perspect. Med.* **2022**, *12*, a041178.
[60] H. Wiig, D. Keskin, R. Kalluri, *Matrix Biol.* **2010**, *29*, 645.
[61] G. Baranwal, H. A. Creed, W. E. Cromer, W. Wang, B. D. Upchurch, M. C. Smithhart, S. S. Vadlamani, M.-C. C. Clark, N. C. Busbuso, S. N. Blais, A. J. Reyna, R. M. Dongaonkar, D. C. Zawieja, J. M. Rutkowski, *Acta Physiol.* **2021**, *232*, e13656.
[62] W. Zheng, H. Nurmi, S. Appak, A. Sabine, E. Bovay, E. A. Korhonen, F. Orsenigo, M. Lohela, G. D'amico, T. Holopainen, C. C. Leow, E. Dejana, T. V. Petrova, H. G. Augustin, K. Alitalo, *Genes Dev.* **2014**, *28*, 1592.
[63] F. Zhang, G. Zarkada, J. Han, J. Li, A. Dubrac, R. Ola, G. Genet, K. Boyé, P. Michon, S. E. Künzel, J. P. Camporez, A. K. Singh, G.-H. Fong, M. Simons, P. Tso, C. Fernández-Hernando, G. I. Shulman, W. C. Sessa, A. Eichmann, *Science* **2018**, *361*, 599.
[64] J. W. Breslin, S. Y. Yuan, M. H. Wu, *Lymphatic Res. Biol.* **2007**, *5*, 105.
[65] C. Tacconi, C. Correale, A. Gandelli, A. Spinelli, E. Dejana, S. D'Alessio, S. Danese, *Gastroenterology* **2015**, *148*, 1438.
[66] C. Norrmén, K. I. Ivanov, J. Cheng, N. Zangger, M. Delorenzi, M. Jaquet, N. Miura, P. Puolakkainen, V. Horsley, J. Hu, H. G. Augustin, S. Ylä-Herttuala, K. Alitalo, T. V. Petrova, *J. Cell Biol.* **2009**, *185*, 439.
[67] S. Schulte-Merker, A. Sabine, T. V. Petrova, *J. Cell Biol.* **2011**, *193*, 607.
[68] A. Sabine, Y. Agalarov, H. Maby-El Hajjami, M. Jaquet, R. Hägerling, C. Pollmann, D. Bebber, A. Pfenniger, N. Miura, O. Dormond, J.-M. Calmes, R. H. Adams, T. Mäkinen, F. Kiefer, B. R. Kwak, T. V. Petrova, *Dev. Cell* **2012**, *22*, 430.
[69] D. Jiang, J. Liang, P. W. Noble, *Physiol. Rev.* **2011**, *91*, 221.
[70] D. G. Jackson, *Matrix Biol.* **2019**, *78-79*, 219.
[71] L. A. Johnson, S. Banerji, W. Lawrance, U. Gileadi, G. Prota, K. A. Holder, Y. M. Roshorm, T. Hanke, V. Cerundolo, N. W. Gale, D. G. Jackson, *Nat. Immunol.* **2017**, *18*, 762.
[72] J. Bernier-Latmani, T. V. Petrova, *Nat. Rev. Gastroenterol. Hepatol.* **2017**, *14*, 510.
[73] J. Bernier-Latmani, C. Cisarovsky, C. S. Demir, M. Bruand, M. Jaquet, S. Davanture, S. Ragusa, S. Siegert, O. Dormond, R. Benedito, F. Radtke, S. A. Luther, T. V. Petrova, *J. Clin. Invest.* **2015**, *125*, 4572.
[74] M.-L. Bochaton-Piallat, G. Gabbiani, B. Hinz, *F1000Research* **2016**, *5*, 752.
[75] M. Pehrsson, J. H. Mortensen, T. Manon-Jensen, A. C. Bay-Jensen, M. A. Karsdal, M. J. Davies, *Expert Rev. Mol. Diagn.* **2021**, *21*, 1049.
[76] I. V. Martin, E. Borkham-Kamphorst, S. Zok, C. R. C. Van Roeyen, U. Eriksson, P. Boor, K. Hittatiya, H.-P. Fischer, H. E. Wasmuth, R. Weiskirchen, F. Eitner, J. Floege, T. Ostendorf, *Am. J. Pathol.* **2013**, *182*, 107.
[77] L. A. Murray, A. Rubinowitz, E. L. Herzog, *Curr. Opin. Rheumatol.* **2012**, *24*, 656.
[78] Y. Shi, Y. Wang, Q. Li, K. Liu, J. Hou, C. Shao, Y. Wang, *Nat. Rev. Nephrol.* **2018**, *14*, 493.
[79] J. D. Imig, M. J. Ryan, *Compr. Physiol.* **2013**, *3*, 957.
[80] H. Wei, L. Chen, Q. Li, X. Liang, K. Wang, Y. Zhang, Y. Li, Y. Liu, G. Xu, *Int. J. Biol. Sci.* **2022**, *18*, 1171.
[81] K. W. Tan, S. Z. Chong, F. H. S. Wong, M. Evrard, S. M.-L. Tan, J. Keeble, D. M. Kemeny, L. G. Ng, J.-P. Abastado, V. Angeli, *Blood* **2013**, *122*, 3666.
[82] A.-K. Meinecke, N. Nagy, G. D.'. Lago, S. Kirmse, R. Klose, K. Schrödter, A. Zimmermann, I. Helfrich, H. Rundqvist, D. Theegarten, O. Anhenn, V. Orian-Rousseau, R. S. Johnson, K. Alitalo, J. W. Fischer, J. Fandrey, C. Stockmann, *Blood* **2012**, *119*, 5931.
[83] F. Ogata, K. Fujiu, S. Matsumoto, Y. Nakayama, M. Shibata, Y. Oike, I. Koshima, T. Watabe, R. Nagai, I. Manabe, *J. Invest. Dermatol.* **2016**, *136*, 706.
[84] M. Baues, A. Dasgupta, J. Ehling, J. Prakash, P. Boor, F. Tacke, F. Kiessling, T. Lammers, *Adv. Drug Delivery Rev.* **2017**, *121*, 9.

[85] C. J. Philp, I. Siebeke, D. Clements, S. Miller, A. Habgood, A. E. John, V. Navaratnam, R. B. Hubbard, G. Jenkins, S. R. Johnson, *Am. J. Respir. Cell Mol. Biol.* **2018**, *58*, 594.
[86] K. R. Levental, H. Yu, L. Kass, J. N. Lakins, M. Egeblad, J. T. Erler, S. F. T. Fong, K. Csiszar, A. Giaccia, W. Weninger, M. Yamauchi, D. L. Gasser, V. M. Weaver, *Cell* **2009**, *139*, 891.
[87] Y. J. Cha, J. H. Youk, B. G. Kim, W. H. Jung, N. H. Cho, *Yonsei Med. J.* **2016**, *57*, 599.
[88] H. Kinashi, N. Toda, T. Sun, T. Q. Nguyen, Y. Suzuki, T. Katsuno, H. Yokoi, J. Aten, M. Mizuno, S. Maruyama, M. Yanagita, R. Goldschmeding, Y. Ito, *Sci. Rep.* **2019**, *9*, 12175.
[89] T. A. Wynn, *Nat. Rev. Immunol.* **2004**, *4*, 583.
[90] T. Avraham, S. Daluvoy, J. Zampell, A. Yan, Y. S. Haviv, S. G. Rockson, B. J. Mehrara, *Am. J. Pathol.* **2010**, *177*, 3202.
[91] T. Avraham, J. C. Zampell, A. Yan, S. Elhadad, E. S. Weitman, S. G. Rockson, J. Bromberg, B. J. Mehrara, *FASEB J.* **2013**, *27*, 1114.
[92] N. W. Clavin, T. Avraham, J. Fernandez, S. V. Daluvoy, M. A. Soares, A. Chaudhry, B. J. Mehrara, *Am. J. Physiol.: Hear. Circ. Physiol.* **2008**, *295*, H2113.
[93] T. Avraham, S. Daluvoy, J. Zampell, A. Yan, Y. S. Haviv, S. G. Rockson, B. J. Mehrara, *Am. J. Pathol.* **2010**, *177*, 3202.
[94] R. C. Ji, *Cancer Lett.* **2014**, *346*, 6.
[95] X. Geng, Y. C. Ho, R. S. Srinivasan, *Cell. Mol. Life Sci.* **2021**, *78*, 5903.
[96] L. V. Leak, J. F. Burke, *J. Cell Biol.* **1968**, *36*, 129.
[97] C. Danussi, P. Spessotto, A. Petrucco, B. Wassermann, P. Sabatelli, M. Montesi, R. Doliana, G. M. Bressan, A. Colombatti, *Mol. Cell. Biol.* **2008**, *28*, 4026.
[98] A. Capuano, E. Pivetta, F. Baldissera, G. Bosisio, B. Wassermann, F. Bucciotti, A. Colombatti, P. Sabatelli, R. Doliana, P. Spessotto, *Matrix Biol.* **2019**, *81*, 34.
[99] B. G. Coon, N. Baeyens, J. Han, M. Budatha, T. D. Ross, J. S. Fang, S. Yun, J.-L. Thomas, M. A. Schwartz, *J. Cell Biol.* **2015**, *208*, 975.
[100] T. Makinen, *EMBO J.* **2001**, *20*, 4762.
[101] D. O. Miteva, J. M. Rutkowski, J. B. Dixon, W. Kilarski, J. D. Shields, M. A. Swartz, *Circ. Res.* **2010**, *106*, 920.
[102] J. Kazenwadel, K. L. Betterman, C.-E. Chong, P. H. Stokes, Y. K. Lee, G. A. Secker, Y. Agalarov, C. S. Demir, D. M. Lawrence, D. L. Sutton, S. P. Tabruyn, N. Miura, M. Salminen, T. V. Petrova, J. M. Matthews, C. N. Hahn, H. S. Scott, N. L. Harvey, *J. Clin. Invest.* **2015**, *125*, 2979.
[103] W.-C. Huang, M. Nagahashi, K. Terracina, K. Takabe, *Biomolecules* **2013**, *3*, 408.
[104] M. Frye, A. Taddei, C. Dierkes, I. Martinez-Corral, M. Fielden, H. Ortsäter, J. Kazenwadel, D. P. Calado, P. Ostergaard, M. Salminen, L. He, N. L. Harvey, F. Kiefer, T. Mäkinen, *Nat. Commun.* **2018**, *9*, 1511.
[105] D. T. Sweet, J. M. Jiménez, J. Chang, P. R. Hess, P. Mericko-Ishizuka, J. Fu, L. Xia, P. F. Davies, M. L. Kahn, *J. Clin. Invest.* **2015**, *125*, 2995.
[106] J. Kazenwadel, K. L. Betterman, C.-E. Chong, P. H. Stokes, Y. K. Lee, G. A. Secker, Y. Agalarov, C. S. Demir, D. M. Lawrence, D. L. Sutton, S. P. Tabruyn, N. Miura, M. Salminen, T. V. Petrova, J. M. Matthews, C. N. Hahn, H. S. Scott, N. L. Harvey, *J. Clin. Invest.* **2015**, *125*, 2979.
[107] L. Alderfer, E. Russo, A. Archilla, B. Coe, D. Hanjaya-Putra, *FASEB J.* **2021**, *35*, e21498.
[108] F. Liu, D. Lagares, K. M. Choi, L. Stopfer, A. Marinković, V. Vrbanac, C. K. Probst, S. E. Hiemer, T. H. Sisson, J. C. Horowitz, I. O. Rosas, L. E. Fredenburgh, C. Feghali-Bostwick, X. Varelas, A. M. Tager, D. J. Tschumperlin, *Am. J. Physiol.: Lung Cell. Mol. Physiol.* **2015**, *308*, L344.
[109] T. Kariya, H. Nishimura, M. Mizuno, Y. Suzuki, Y. Matsukawa, F. Sakata, S. Maruyama, Y. Takei, Y. Ito, *Am. J. Physiol.* **2018**, *314*, F167.
[110] M. Oka, C. Iwata, H. I. Suzuki, K. Kiyono, Y. Morishita, T. Watabe, A. Komuro, M. R. Kano, K. Miyazono, *Blood* **2008**, *111*, 4571.
[111] H. Kinashi, Y. Ito, M. Mizuno, Y. Suzuki, T. Terabayashi, F. Nagura, R. Hattori, Y. Matsukawa, T. Mizuno, Y. Noda, H. Nishimura, R. Nishio, S. Maruyama, E. Imai, S. Matsuo, Y. Takei, *J. Am. Soc. Nephrol.* **2013**, *24*, 1627.
[112] A. S. Lee, J. E. Lee, Y. J. Jung, D. H. Kim, K. P. Kang, S. Lee, S. K. Park, S. Y. Lee, M. J. Kang, W. S. Moon, H. J. Kim, Y. B. Jeong, M. J. Sung, W. Kim, *Kidney Int.* **2013**, *83*, 50.
[113] Y. Suzuki, Y. Ito, M. Mizuno, H. Kinashi, A. Sawai, Y. Noda, T. Mizuno, H. Shimizu, Y. Fujita, K. Matsui, S. Maruyama, E. Imai, S. Matsuo, Y. Takei, *Kidney Int.* **2012**, *81*, 865.
[114] A. R. Henderson, I. S. Ilan, E. Lee, *Microcirculation* **2021**, *28*, e12730.
[115] W. J. Polacheck, M. L. Kutys, J. B. Tefft, C. S. Chen, *Nat. Protoc.* **2019**, *14*, 1425.
[116] E. Akbari, G. B. Spychalski, K. K. Rangharajan, S. Prakash, J. W. Song, *Lab Chip* **2018**, *18*, 1084.
[117] N. R. Harris, N. R. Nielsen, J. B. Pawlak, A. Aghajanian, K. Rangarajan, D. S Serafin, G. Farber, D. M. Dy, N. P. Nelson-Maney, W. Xu, D. Ratra, S. H. Hurr, L. Qian, J. P. Scallan, K. M. Caron, *Circ. Res.* **2022**, *130*, 5.
[118] H. Kinashi, Y. Ito, T. Sun, T. Katsuno, Y. Takei, *Int. J. Mol. Sci.* **2018**, *19*, 2487.
[119] R. P. Kataru, K. Jung, C. Jang, H. Yang, R. A. Schwendener, J. E. Baik, S. H. Han, K. Alitalo, G. Y. Koh, *Blood* **2009**, *113*, 5650.
[120] J. C. Zampell, A. Yan, S. Elhadad, T. Avraham, E. Weitman, B. J. Mehrara, *PLoS One* **2012**, *7*, e49940.
[121] V. Riabov, A. Gudima, N. Wang, A. Mickley, A. Orekhov, J. Kzhyshkowska, *Front. Physiol.* **2014**, *5*, 75.
[122] E. Brakenhielm, K. Alitalo, *Nat. Rev. Cardiol.* **2019**, *16*, 56.
[123] O. Henri, C. Pouehe, M. Houssari, L. Galas, L. Nicol, F. Edwards-Lévy, J.-P. Henry, A. Dumesnil, I. Boukhalfa, S. Banquet, D. Schapman, C. Thuillez, V. Richard, P. Mulder, E. Brakenhielm, *Circulation* **2016**, *133*, 1484.
[124] K. Maruyama, K. Naemura, K. Yoshihara, K. Imanaka-Yoshida, H. Kurihara, S. Miyagawa-Tomita, *STAR Protoc.* **2021**, *2*, 100775.
[125] R. Ogino, K. Hayashida, S. Yamakawa, E. Morita, *Int. J. Mol. Sci.* **2020**, *21*, 3885.
[126] S. Karaman, M. Detmar, *J. Clin. Invest.* **2014**, *124*, 922.
[127] A. Padoan, M. Plebani, D. Basso, *Int. J. Mol. Sci.* **2019**, *20*, 676.
[128] M. Esquivel-Velázquez, P. Ostoa-Saloma, M. I. Palacios-Arreola, K. E. Nava-Castro, J. I. Castro, J. Morales-Montor, *J. Interferon Cytokine Res.* **2015**, *35*, 1.
[129] B. Piersma, M.-K. Hayward, V. M. Weaver, *Biochim. Biophys. Acta, Rev. Cancer* **2020**, *1873*, 188356.
[130] C.-N. Shen, K.-S. Goh, C.-R. Huang, T.-C. Chiang, C.-Y. Lee, Y.-M. Jeng, S.-J. Peng, H.-J. Chien, M.-H. Chung, Y.-H Chou, C.-C. Hsieh, S. Kulkarni, P. J. Pasricha, Y.-W. Tien, S.-C. Tang, *EBio-Medicine* **2019**, *47*, 98.
[131] B. Rybinski, J. Franco-Barraza, E. Cukierman, *Physiol. Genomics* **2014**, *46*, 223.
[132] M. D. Donnan, Y. Kenig-Kozlovsky, S. E. Quaggin, *Nat. Rev. Nephrol.* **2021**, *17*, 655.
[133] M. Lech, H.-J. Anders, *Biochim. Biophys. Acta, Mol. Basis Dis.* **2013**, *1832*, 989.
[134] Y. Wang, J. Wang, C. Yang, Y. Wang, J. Liu, Z. Shi, Y. Chen, Y. Feng, X. Ma, S. Qiao, *World J. Surg. Oncol.* **2021**, *19*, 91.
[135] H. Kurahara, S. Takao, K. Maemura, Y. Mataki, T. Kuwahata, K. Maeda, M. Sakoda, S. Iino, S. Ishigami, S. Ueno, H. Shinchi, S. Natsugoe, *Pancreas* **2013**, *42*, 155.
[136] H. Kurahara, H. Shinchi, Y. Mataki, K. Maemura, H. Noma, F. Kubo, M. Sakoda, S. Ueno, S. Natsugoe, S. Takao, *J. Surg. Res.* **2011**, *167*, e211.

[137] B. Zhang, G. Yao, Y. Zhang, J. Gao, B. Yang, Z. Rao, J. Gao, *Clinics* **2011**, *66*, 1879.
[138] Y. Zhang, C. Zhang, L. Li, X. Liang, P. Cheng, Q. Li, X. Chang, K. Wang, S. Huang, Y. Li, Y. Liu, G. Xu, *Cell Death Dis.* **2021**, *12*, 109.
[139] M. Cimini, A. Cannatá, G. Pasquinelli, M. Rota, P. Goichberg, *PLoS One* **2017**, *12*, e0173927.
[140] L. Greiwe, M. Vinck, F. Suhr, *Acta Physiol.* **2016**, *217*, 61.
[141] R. M. Dongaonkar, R. H. Stewart, H. J. Geissler, G. A. Laine, *Cardiovasc. Res.* **2010**, *87*, 331.
[142] U. Mehlhorn, H. J. Geissler, G. A. Laine, S. J. Allen, *Eur. J. Cardio-Thorac. Surg.* **2001**, *20*, 1220.
[143] M. B. Aldrich, E. M. Sevick-Muraca, *Cytokine* **2013**, *64*, 362.
[144] R. P. Kataru, K. Jung, C. Jang, H. Yang, R. A. Schwendener, J. E. Baik, S. H. Han, K. Alitalo, G. Y. Koh, *Blood* **2009**, *113*, 5650.
[145] W. Zheng, A. Aspelund, K. Alitalo, *J. Clin. Invest.* **2014**, *124*, 878.
[146] A. M. Kolarzyk, G. Wong, E. Lee, *Cold Spring Harbor Perspect. Med.* **2022**, *12*, a041169.
[147] L. Alderfer, E. Hall, D. Hanjaya-Putra, *Acta Biomater.* **2021**, *133*, 34.
[148] S. Blacher, C. Erpicum, B. Lenoir, J. Paupert, G. Moraes, S. Ormenese, E. Bullinger, A. Noel, *PLoS One* **2014**, *9*, e97019.
[149] J. W. Robering, A. Weigand, R. Pfuhlmann, R. E. Horch, J. P. Beier, A. M. Boos, *J. Cell. Mol. Med.* **2018**, *22*, 3740.
[150] H. Kwon, J.-Y. Kwon, J. Song, Y.-S. Maeng, *Int. J. Mol. Sci.* **2021**, *22*, 4237.
[151] S. Landau, A. Newman, S. Edri, I. Michael, S. Ben-Shaul, Y. Shandalov, T. Ben-Arye, P. Kaur, M. H. Zheng, S. Levenberg, *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2101931118.
[152] A. R. Henderson, H. Choi, E. Lee, *Micromachines* **2020**, *11*, 147.
[153] K. Haase, R. D. Kamm, *Regener. Med.* **2017**, *12*, 285.
[154] S. R. Moses, J. J. Adorno, A. F. Palmer, J. W. Song, *Am. J. Physiol.: Cell Physiol.* **2020**, *320*, C92.
[155] J. W. Song, L. L. Munn, *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 15342.
[156] N. Ahmadzadeh, J. W. Robering, A. Kengelbach-Weigand, M. Al-Abboodi, J. P. Beier, R. E. Horch, A. M. Boos, *Exp. Cell Res.* **2020**, *388*, 111816.
[157] S. Kim, M. Chung, N. L. Jeon, *Biomaterials* **2016**, *78*, 115.
[158] H. Duong, B. Wu, B. Tawil, *Tissue Eng., Part A* **2009**, *15*, 1865.
[159] H. Zhuo, D. Zhou, Y. Wang, H. Mo, Y. Yu, Y. Liu, *Am. J. Physiol.* **2019**, *317*, F1022.
[160] L. Alderfer, A. Wei, D. Hanjaya-Putra, *J. Biol. Eng.* **2018**, *12*, 32.
[161] E. Solari, C. Marcozzi, D. Negrini, A. Moriondo, *Exp. Biol. Med.* **2020**, *9*, 463.
[162] E. Gordon, L. Schimmel, M. Frye, *Front. Physiol.* **2020**, *11*, 684.
[163] V. Triacca, E. Güç, W. W. Kilarski, M. Pisano, M. A. Swartz, *Circ. Res.* **2017**, *120*, 1440.
[164] W. Y. Wang, D. Lin, E. H. Jarman, W. J. Polacheck, B. M. Baker, *Lab Chip* **2020**, *20*, 1153.

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