Review

Cellular Chirality and Cellular Tissue Indexes in Vascular Morphogenesis

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Cellular Chirality and Cellular Tissue Indexes in Vascular Morphogenesis

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Abstract: During embryonic development, angiogenesis and arteriogenesis are responsible for vast growth and remodeling. These processes have distinct mechanisms, like budding, cord hollowing, cell hollowing, cell wrapping, and intussusception. This review discusses the diversity of morphogenetic mechanisms contributing to vessel assembly and angiogenic sprouting in blood vessels and how molecular pathways regulate some complex cell behaviors concerning the VEGFR pathway. Also, a particular part is dedicated to the HIF 1α gene. The VEGFR pathway's key components are VEGF receptors VEGFR1, VEGFR2, and VEGFR3. VEGFR2 plays a central role in vascular morphogenesis. VEGF is the primary ligand involved in angiogenesis and arteriogenesis. Various types of VEGF are in study regarding their therapeutic use. The ultimate goal of the vascular morphogenesis study is to enable the development of organized vascular tissue that presumably might be used to replace the disses-affected one. Cellular chirality—the intrinsic "handedness" of cells in movement, structure, and organization—plays a crucial role in angiogenesis, the process by which new blood vessels develop from old ones. This chiral activity is essential for the directed and patterned organization of endothelial cells during vascular formation and remodeling. In angiogenesis, cellular chirality directs endothelial cells to assume specific orientations and migratory patterns, crucial for the formation of functionally organized blood arteries that provide tissues with needed nutrients and oxygen. Cellular chirality in this environment is affected by multiple mechanisms, including VEGF/VEGFR signaling, mechanical pressures, interactions with the extracellular matrix (ECM), and cytoskeletal movements. Lately, researchers have focused on the molecular control of blood vessel morphogenesis, the study of signaling circuitry implied in vascular morphogenesis, the emerging mechanism of vascular stabilization, and helical vasculogenesis driven by cell chirality.

Keywords: angiogenesis; arteriogenesis; vascular morphogenesis; VEGFR; cellular chirality

1. Introduction

The number of papers published involving vascular morphogenesis (which was used as a search key word) has increased exponentially. With only one article published in 1948, over the last five years, the number of documents published in PubMed exceeds 2689 (date of accession 05.10.2023. In this review, cellular and tissue indices are discussed in the literature on vascular morphogenesis. Data regarding this review were curated using the Pubmed database. The words used to generate the data were vascular morphogenesis, cellular indices, and tissular indices.

As expected, the amount of data generated is considerable. The ultimate goal of the vascular morphogenesis study is to enable the development of organized vascular tissue that presumably might be used to replace the disease-affected one [1,2].

Growing new vessels requires a coordinated cellular response to growth. This response is sensed and triggered by cell surface receptors responsible for activating an intracellular cascade that initiates migration and controls cell growth. While the dominant molecular mechanisms have been determined, the distinct and detailed interactions remain unknown [3].

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Experiments were performed using mainly mice and zebrafish models. A series of pathways have been identified. These pathways include VEGF-VEGFR, Notch-DSL, Tie-Angiopoietin, VE-cadherin and Ephrin-Eph. Other signaling pathways contributing to further vascular remodeling include plexins, TGF-beta, PDGF, and integrins [4]. In the following paragraphs, the VEGFR pathway, together with its receptors, is briefly discussed.

2. VEGFR Signaling Pathway in Vascular Morphogenesis

Coordinated cellular response is critical for the development of new blood vessels. The cellular response implies the cell surface receptors. Even if the dominant molecular motifs have been determined, how they interact is still not understood entirely [5].

The dominant pathways are the VEGF-VEGFR, Notch-DSL, Tie-Angiopoietin, VE-cadherin, and Ephrin-Eph [6,7].

The VEGF pathway is one of the critical regulators of the angiogenesis process. The VEGF/VEGF-receptor axis comprises multiple ligands and receptors with overlapping and distinct ligand-receptor binding specificities. The VEGF-receptor pathway activates signaling processes that promote endothelial cell growth, migration, and survival from preexisting vasculature. In addition, VEGF mediates vessel permeability [8].

VEGF is crucial in vasculogenesis and angiogenesis. VEGF activity is restricted mainly to vascular endothelium cells, although it does affect other cell types (e.g., stimulation monocyte/macrophage migration). In vitro, VEGF stimulates endothelial cell mitogenesis and cell migration [9].

All members of the VEGF molecular family determine a cellular response by binding to tyrosine kinase receptors (the VEGFRs) on the cell surface, causing a dimerization and activation through transphosphorylation [10].

Five VEGF molecules are known: VEGF A-E. VEGFA binds to VEGFR1 (Flt-1) and VEGFR -2 (Flk-1/KDR). VEGF-B stimulates VEGFR1 (Flt-1). VEGF-C interacts with VEGFR -2 (Flk-1/KDR) and VEGFR-3(Flt-4). VEGF-D stimulates VEGFR -2 (Flk-1/KDR) and VEGFR-3(Flt-4). Lastly, VEGFR-E enables VEGFR -2 (Flk-1/KDR)[11–15]Figure 1).

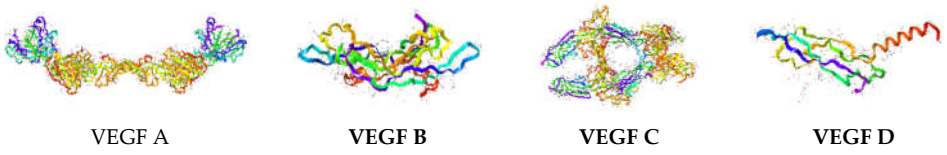

Figure 1. Vascular endothelial growth factors from A-D are represented as ribbons.

The VEGFA gene encodes VEGF-A—a platelet-derived growth factor (PDGF)/vascular endothelial growth factor (VEGF) family member. The protein encoded is a disulfide-linked homodimer [16,17].

The Vascular Endothelial Growth Factor (VEGF) signaling pathway is essential for angiogenesis, the formation of new blood vessels from pre-existing ones. VEGF attaches to its receptors on endothelial cells, chiefly VEGFR1 (Flt-1), VEGFR2 (K.D.R./Flk-1), and VEGFR3 (Flt-4), triggering a series of internal processes that govern endothelial cell activity. The principal steps of the VEGF-VEGFR pathway are as follows: (a) The binding of VEGF to VEGFRs: VEGF-A is the primary isoform of VEGF implicated in angiogenesis. It exhibits a high affinity for both VEGFR1 and VEGFR2, triggering receptor dimerization and activation.(b) Activation of VEGFRs: Following VEGF binding, VEGFRs experience auto-phosphorylation at certain tyrosine residues in their intracellular domains, resulting in the recruitment and activation of downstream signaling molecules.Ownstream Signaling Routes: The PI3K/Akt pathway is initiated when activated VEGFRs recruit phosphoinositide 3-kinase (PI3K), resulting in the synthesis of phosphatidylinositol (3,4,5)-trisphosphate (PIP3). This stimulates protein kinase B (Akt), which governs cell survival, proliferation, and migration. The activation of VEGFR also initiates the mitogen-activated protein kinase (MAPK) pathway, encompassing

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extracellular signal-regulated kinase (ERK). ERK signaling modulates endothelial cell proliferation and migration. Regulation of Gene Expression: The activation of VEGFRs and subsequent signaling pathways results in alterations in gene expression, notably the upregulation of pro-angiogenic factors such as endothelial nitric oxide synthase (eNOS) and matrix metalloproteinases (MMPs), which facilitate endothelial cell proliferation, migration, and tube formation. (e) Responses of Endothelial Cells: The signaling processes initiated by the VEGF-VEGFR connection promote endothelial cell proliferation, migration, and survival, resulting in the development of new blood vessels.Negative Regulation: The VEGF-VEGFR pathway is stringently controlled by multiple mechanisms, including the function of soluble VEGFRs (sVEGFR1 and sVEGFR2) that serve as decoy receptors, sequestering VEGF and inhibiting its interaction with membrane-bound VEGFRs. Role of Co-Receptors: Neuropilins (NRP1 and NRP2) function as co-receptors for VEGFRs and regulate VEGF signaling. They augment VEGF affinity for VEGFR2 and modulate VEGF-mediated angiogenesis [18,19]. In the following, some relevant VEGFR 1-3 are described briefly.

2.1. VEGFR1

Expression of VEGFR1 depends on the microenvironment, which is distinctively regulated under hypoxic and inflammatory conditions. VEGFR1 activation can affect vascular permeability and induce macrophage and microglia production of proinflammatory and pro-angiogenic mediators. Also, the ability of the VEGFR1 ligands (VEGF-A, PlGF, VEGF-B) to compete against each other for receptor binding and to heterodimerize is remarkable. Clinically, anti-VEGF drugs have proven effective in proliferative diseases, and their impact on the modulation of VEGFR1 signaling is still an opportunity for further research [20]. VEGFR1 may have a role as a negative regulator of embryonic angiogenesis. It promotes the PGF-mediated proliferation of endothelial cells. It has a very high affinity for VEGFA and relatively low protein kinase activity. Also, it functions as a negative regulator of VEGFA signaling. Furthermore, VEGFR1 stimulation led to phosphorylation of the regulatory subunit of phosphatidylinositol 3-kinase (PIK3R1). VEGFR1 is also implied in the activation of MAPK1/ERK2, MAPK3/ERK1, the M.A.P. kinase signaling pathway, and the AKT1 signaling pathway [21].

VEGFR1 promotes endothelial tubule branching in an organotypic angiogenesis model via a mechanism that requires Rab4A and alphavbeta3 Integrin. A recycling pathway regulated by Rab4A is an effector of VEGFR1 during the branching morphogenesis of the vasculature [22]. The VEGFR1 gene encodes a receptor tyrosine kinase and a secreted splice variant. Epidermal growth factor /fibroblast growth factor two (EGF/FGF2)-mediated VEGFR1 induction is mediated via the functional interaction of transcription factors ETS1 and hypoxia-inducible factor 2 alpha(HIF-2alpha). Mechanistic analyses revealed that EGF/FGF2 signaling induces ETS1 expression in endothelial cells, increases HIF-2alpha protein level without hypoxia, and recruits both protein C-ets-1(ETS1) and HIF-2alpha to the VEGFR1 chromatin domain [23]. VEGFA regulates embryonic angiogenesis through vascular endothelial growth factor receptor 2 (VEGFR2) expressed in the endocardium. It is shown that VEGFR1 produced in the endocardium negatively regulates embryonic coronary angiogenesis by limiting the Vegf-Notch signaling [24]. VEGFR1 and VEGFR2 expressions are upregulated during copy number variation (C.N.V.) pathogenesis. Both MF1 and DC101 significantly suppressed C.N.V. at 50 mg/kg [25]. The role of VEGF receptor 1 (VEGFR1) signaling in angiogenesis and tissue growth in an endometriosis model showed that VEGFR1 is implied in this process. VEGFR1 signaling in host-derived cells is essential for cell growth [26,27]. VEGFR1 exists in different forms, resulting from alternative splicing of the same gene. Moreover, sVEGFR-1 and sFlt1-14 are additional in angiogenesis [28]. A recycling pathway regulated by Rab4A is a critical effector of VEGFR1 during the branching morphogenesis of the vasculature [29]. Tyrosine-protein kinase acts as a cell-surface receptor for VEGFA, VEGFC, and VEGFD. The binding of vascular growth factors to isoform one leads to the activation of several signaling cascades. Activation of PLCG1 stimulates the production of the cellular signaling molecules diacylglycerol and inositol 1,4,5-trisphosphateConsecutivelly they stimulate protein kinase C [30]. Angiogenesis stimulates endothelial cells (E.C.) by various cytokines and growth factors [31]. The biological effects of VEGF are mediated by two tyrosine kinase receptors,

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Flt-1 (VEGFR-1) and K.D.R. (VEGFR-2). VEGF is essential for the early development of the vasculature [32].

The properties of VEGFR1 are summarized in Table 1:

Table 1. VEGFR1 properties.

#	Property	Description	References
1	Structure	VEGFR1 is a transmembrane protein belonging to the receptor tyrosine kinase (R.T.K.) family.	[33]
2	Function	VEGFR1 primarily functions as a receptor for VEGF-A.	[34]
3	Binding Affinity	VEGFR1 has a high affinity for VEGF-A.	[35]
4	Role in Development	VEGFR1 plays a critical role in the formation of the vascular system during embryonic development.	[36]
5	Signal Transduction	Upon activation, VEGFR1 undergoes autophosphorylation and activates downstream signaling pathways.	[37]
6	Angiogenesis Regulation	VEGFR1 is involved in the negative regulation of angiogenesis.	[38]
7	Therapeutic Target	VEGFR1 has been explored as a therapeutic target for antiangiogenic drugs.	[39]
8	Soluble Form	VEGFR1 can exist in a soluble form (sVEGFR1) due to alternative splicing, acting as a decoy receptor for VEGF-A.	[40]
9	Expression in Cancer	VEGFR1 expression is observed in various cancers and is associated with tumor angiogenesis, progression, and poor prognosis.	[41]
10	Interaction with Neuropilin-1	VEGFR1 can form a complex with neuropilin-1, enhancing VEGF-A binding and signaling.	[42]
11	Regulation by miRNAs	VEGFR1 expression can be regulated by microRNAs (miRNAs) in various physiological and pathological conditions.	[43]
12	Role in Neuroprotection	VEGFR1 has been implicated in neuroprotection and neuronal survival in addition to its role in angiogenesis.	[44]

In the following, VEGFR2, a fundamental structure in the vasculogenesis process, is discussed.

2.2. VEGFR2

VEGFR-2 is a 210-230 kDa glycoprotein expressed in vascular endothelial cells and binds VEGF-A. VEGFR-2 is closely related to VEGFR-1, for they have expected and specific ligands. Furthermore, VEGFR-2 is a highly active kinase in contrast with VEGFR1, an impaired tyrosine kinase receptor. The signaling pathways, Y1175 and Y1214, are the main autophosphorylation sites of the human VEGFR-2 when VEGF is bound [45–47].

VEGFR-2 is a part of the VEGF family. It is essential for developmental and reparative angiogenesis [48]. VEGF activates VEGFR2, situated in the endothelial cell membrane. VEGFR2 interacts with VRASP(), PLCγ, ScK, Cdc42, Src, and subsequently PI3K. VRAP and Sck do not engage in further interactions with other signaling molecules. PLCγ interacts with diacylglycerol and inositol trisphosphate. Cdc42 engages with p38. PI3K activates PIP3. IP3 engages with Ca+ and PKC, initiating a cascade in which SPK connects with Ras, and then engaging with Raf-1, so boosting M.E.K., which interacts with ERK. ErK enhances vascular proliferation through its interaction with DNA. Calcium ions (Ca+) are activated by inositol trisphosphate (IP3), which then engages with calnexin (CALN), facilitating the generation of nitric oxide synthase (NFTA) that interacts with cyclooxygenase-2 (COX2); the COX2 pathway subsequently triggers the synthesis of prostaglandin I2 (PGI2). FAK activation subsequently activates focal adhesion and cellular migration. Additionally,

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p38 activates M.A.P., thereby enhancing actin rearrangement through its interaction with HSP27, ultimately resulting in cell migration. Additionally, AktPKE activates eNos, Casp9, and Bad, which further enhances cell permeability, migration, and survival. Numerous studies indicate that VEGFR-2 is the principal mediator of VEGF-induced responses in endothelial cells. It serves as a vital signal transducer in both physiological and pathological angiogenesis [49,50].

Regarding the Notch signaling pathway, Delta notch or Seratt-like ligand, with the help of O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase (Fringe), stimulates the Notch receptor that further promotes the Notch intracellular domain. The Notch domain simulation acts on D.N.A. that additionally produces Hes 1/5(hes family bHLH transcription factor 1), Hey(hes related family bHLH transcription factor with YRPW motif 1), PreTα(pre T cell antigen receptor alpha), and NRAP(Notch-regulated ankyrin repeat-containing protein) respectively. On C.S.L. (recombination signal binding protein for immunoglobulin kappa J region like) act a series of activators -MAML, H.A.T.s, SKIP and a series of Co suppressors- SMRT, CtBP, Gro/TLE, C.I.R., SHARP, HDAC, ATXN1L, Hes1, and NRARP respectively. The Notch signaling pathway is crucial for proper embryonic development [51].

VEGF exerts biological effects through specific receptors on endothelial cell surfaces, with VEGFA/VEGFR2 signaling prominently mediating cellular responses in angiogenesis. Although weaker, VEGF/VEGFR1 signaling converges to the VEGFR2 pathway, activating multiple downstream pathways that promote endothelial cell proliferation [52]. VEGFR2-dependent activation of PI3K-AKT-mTOR signaling regulates cell survival, proliferation, anti-apoptotic functions, and cell permeability. Another crucial VEGF-mediated cell proliferation pathway involves PLCI-mediated activation of PKC, leading to downstream induction of the ERK pathway and another PKC-dependent pathway. Endothelial cell migration is influenced by VEGFA/VEGFR2 signaling through activation of p38MAPK (actin polymerization) necessary in directed migration [53].The VEGFA/VEGFR2 signaling network compiles literature data, especially regarding VEGFA-165, through VEGFR2 in endothelial cells [54]. Signaling molecules—categorized into enzymes, receptors, and transcription factors, undergo contextual activation/deactivation downstream of VEGFA/VEGFR2 signaling, influencing angiogenesis. The interactive VEGFA/VEGFR2 signaling network has a critical role in [55,56]. FAK regulates VEGFR2 and several other angiogenesis-related genes while influencing VEGFR2 and VEGF protein expression in TNBC cells [57]. Despite efforts, clinical success in promoting sprouting angiogenesis in the skeletal muscles of individuals with peripheral artery disease has not been achieved [58].

Vascular endothelial growth factor-A (VEGF-A) is essential for endothelial cell functions associated with angiogenesis. Signal transduction networks initiated by VEGFA/VEGFR2, the most prominent ligand-receptor complex in the VEGF system, lead to endothelial cell proliferation, migration, survival, and new vessel formation involved in angiogenesis [59]. Cell signaling governs cellular behavior and is subject to tight spatiotemporal regulation. Signaling output is modulated by specialized cell membranes and vesicles containing unique lipids and protein combinations. The phosphatidylinositol 4,5-bisphosphate (P.I. (4,5)P2), an essential component of the plasma membrane and other subcellular membranes, is involved in multiple cellular processes [60]. Rap1a and Rap1b, two highly homologous small G proteins, are required for angiogenesis in vivo and normal E.C. responses to VEGF. These results provide an insight into the role of Rap1 in VEGF signaling in E.C.s [61].

Coronary vessels in embryonic mouse hearts originate from various progenitor populations, including sinus venosus (S.V.), endocardium, and proepicardium [62]. Despite the unknown role of hypoxia and its downstream signaling in coronary vessel development, some studies have explored this aspect, identifying SOX17- and VEGF-R2-mediated signaling as potential downstream pathways influenced by hypoxia [63]. S.S.A. inhibits angiogenesis and tumor growth by blocking the VEGFR2-mediated signaling pathway [64]. In response to hypoxia, VEGF promotes angiogenesis by inducing endothelial cell sprouting, proliferation, and migration. Inducing arteriogenesis after cardiac or cerebral arterial occlusion can reduce ischemia and improve disease outcomes, with endothelial VEGF receptor 2 (VEGFR2) signaling governing both processes [65]. Identifying angiogenic factors,

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such as perlecan, in vertebrate development enhances the understanding of the molecular basis of angiogenesis and may inform angiogenesis-based therapeutic approaches [66].

Moreover, focal adhesion kinase (FAK) is essential in embryonic angiogenesis, governing endothelial cell (E.C.) survival and barrier functions via both kinase-independent and -dependent mechanisms. EC-specific tamoxifen-inducible FAK knockout and FAK kinase-defective (K.D.) mutant knockin mice were created to examine the role and kinase activity of FAK in adult angiogenesis. The absence of FAK or its kinase activity diminished endothelial cell proliferation and migration, suggesting that FAK predominantly functions as a kinase in the regulation of adult endothelial cell-mediated angiogenesis. Subsequent research utilizing mouse E.C. line MS1 cells demonstrated that FAK regulates VEGFR2 expression, necessitating both FAK kinase activity and its nuclear translocation.[67]. VEGF, a crucial regulator of angiogenesis, and its receptors, VEGFR1, VEGFR2, and Neuropilin1 (NRP1), are currently targeted in therapeutic strategies for vascular disease. NRP1 is critical in vascular morphogenesis [68]. In the cardiovascular system, VEGF binding to VEGF receptor 2 (VEGFR-2) promotes blood vessel development [69]. However, the activated receptor signals to discrete downstream pathways, coreceptors, and distinct VEGF isoforms modulate the equilibrium between these pathways [70]. Receptor-interacting protein kinase 3 (RIPK3) is a multifunctional intracellular protein recognized as a vital component of the necroptosis-programmed cell death pathway [71]. Some VEGFR2 properties are listed in the table below (Table 2):

Table 2. VEGFR2 properties.

#	Property	Description	References
1	Structure	VEGFR2 is a transmembrane protein belonging to the receptor tyrosine kinase (R.T.K.) family.	[72]
2	Function	VEGFR2 primarily functions as a receptor for VEGF-A, mediating most+*- VEGF-induced angiogenic responses.	[73]
3	Signal Transduction	Upon activation by VEGF-A binding, VEGFR2 undergoes autophosphorylation and activates downstream signaling pathways, including the phosphoinositide 3-kinase (PI3K)/Akt pathway and the mitogen-activated protein kinase (MAPK) pathway.	[74]
4	Angiogenic Response	VEGFR2 activation leads to endothelial cell proliferation, migration, and survival, contributing to angiogenesis.	[75]
5	Role in Development:	VEGFR2 plays a crucial role in embryonic vascular development and angiogenesis.	[76]
6	Regulation of Blood Pressure	VEGFR2 signaling is involved in the regulation of blood pressure and vascular tone.	[77]
7	Therapeutic Target:	VEGFR2 targets antiangiogenic therapy in cancer and other diseases characterized by abnormal angiogenesis.	[78]
8	Endothelial Barrier Function	VEGFR2 signaling is involved in regulating endothelial barrier function, influencing vascular permeability.	.[79]
9	Lymphangiogenesis	VEGFR2 plays a role in lymphangiogenesis, the formation of new lymphatic vessels.	[80]
10	Regulation by miRNAs	VEGFR2 expression can be regulated by microRNAs (miRNAs) in various physiological and pathological conditions	[81]
11	Tie-2 Interaction:	VEGFR2 can form a complex with the Tie-2 receptor, influencing vascular development and stability.	[82]
12	Metastasis Promotion	VEGFR2 signaling has been implicated in promoting tumor metastasis through its effects on tumor vasculature and cancer cell migration.	[83]

VEGFR3's relevant properties are discussed in the following paragraph.

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2.3. VEGFR3

Compared with VEGF-A activation of VEGFR2, VEGF-C-induced VEGFR3 activation led to a more extensive A.K.T. activation, whereas activation of ERK1/2 displayed a distinctly different kinetics [84]. Tyrosine-protein kinase acts as a cell-surface receptor for VEGFC and VEGFD. It plays an essential role in adult lymphangiogenesis and the development of the vascular network and the cardiovascular system during embryonic development. Signaling by activated FLT4 leads to enhanced production of VEGFC [85,86]. Phosphorylation in response to H2O2 is mediated by a process that requires S.R.C. and PRKCD activity. Phosphorylation at Tyr-1068 is required for autophosphorylation at additional tyrosine residues [87,88].

Elevated VEGFR2 activity in postnatal retinas following VEGFR3 deletion or VEGFR3 silencing in cultured endothelial cells reduced vascular endothelial cadherin localization at cell-cell junctions. Simultaneous deletion of VEGFR2 prevented VEGF-induced excessive vascular leakage in Vegfr3-deficient mice. VEGFR3 limits VEGFR2 expression and pathway activity, preventing excessive vascular permeability in quiescent and angiogenic blood vascular endothelial cells [89,90]. The impact of VEGFR3 on lymphatic capillary junctions remains incompletely understood, as excessive VEGFR2 signaling can remodel and seal these junctions [91]. Knockdown of FLT4 in human lymphatic endothelial cells results in impaired NOTCH1 expression and activation, with overexpression of NOTCH1 rescuing button junction formation and interstitial molecule absorption in Flt4 knockout vessels [92–96]. Genetic evidence suggests that VEGFR3 regulates early vessel branching and filopodia formation in the mouse brain, likely mediating the brain vascular phenotype [97–99]. During embryonic development, angiogenesis is initiated as mesoderm mesenchyme cells differentiate into angioblasts expressing VEGFR-2[100–103]. Hypoxia induces angiogenesis, and the injection of VEGFA enhances angiogenesis in animal models. Nonetheless, clinical trials did not reproduce the encouraging outcomes shown in animal models, perhaps due to the methods of delivery and VEGFA's capacity to enhance arterial permeability. Recent trials validate the safety of VEGFA administration in humans, facilitating advancements in pro-angiogenic treatment strategies.[104–108]. Various VEGFA isoforms, including VEGFA111, VEGFA121, VEGFA145, VEGFA148, VEGFA162, and VEGFA165, are commercially available and being tested [109–114].

In the table below, some VEGFR3 properties are summarized (Table 3).

Table 3. VEGFR3 properties.

#	Property	Description	References
1	Structure	VEGFR3 is a transmembrane protein belonging to the receptor tyrosine kinase (R.T.K.) family.	[115]
2	Function	VEGFR3 primarily functions as a receptor for Vascular Endothelial Growth Factor C (VEGF-C) and Vascular Endothelial Growth Factor D (VEGF-D), regulating lymphangiogenesis.	[116]
3	Lymphangiogenesis:	VEGFR3 is a crucial regulator of lymphangiogenesis, the formation of new lymphatic vessels.	[117]
4	Developmental Role	VEGFR3 plays a crucial role in the development of the lymphatic system, including lymphatic vessel sprouting and patterning.	[118]
5	Signal Transduction	Activation of VEGFR3 by its ligands leads to downstream signaling cascades, including the phosphoinositide 3-kinase (PI3K)/Akt pathway and the mitogen-activated protein kinase (MAPK) pathway, regulating lymphatic endothelial cell function.	[119]
6	Role in Cancer Metastasis:	VEGFR3 signaling has been implicated in tumor metastasis by promoting lymphangiogenesis and facilitating cancer cell dissemination through lymphatic vessels.	[120]

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7	Therapeutic Target	Targeting VEGFR3 has been explored as a potential therapeutic strategy for inhibiting lymphangiogenesis and metastasis in cancer.	[121]
8	Interactions with Neuropilins	VEGFR3 can form complexes with neuropilin receptors, modulating its signaling and function in lymphatic endothelial cells.	[122]
9	Regulation by miRNAs:	VEGFR3 expression can be regulated by microRNAs (miRNAs), influencing lymphangiogenesis and cancer progression.	[123]
10	Angiogenesis in Corneal Lymphatics	VEGFR3 plays a role in angiogenesis in corneal lymphatic vessels, influencing corneal inflammation and wound healing.	[124]
11	Role in Lymphedema:	VEGFR3 signaling is implicated in the pathogenesis of lymphedema, providing potential therapeutic targets for its treatment.	[125]
12	Developmental Disorders	Mutations in VEGFR3 are associated with primary lymphedema and other developmental disorders affecting the lymphatic system.	[126]

2.4. Pro-Angiogenic Therapy

Ischemic disease (heart failure, strokes, peripheral artery disease) and also some degenerative are known to be the consequence of ultimately poor blood supply. In this respect, treating these pathologies using pro-angiogenic targeting molecules is tempting. As aspected, VEGFA has the predominant role in vessel formation, growth, and branching [28,127,129–131]. In this respect, VEGFA is the prototype of a pro-angiogenic molecule. VEGFA acts primarily on VEGFR2. Also, due to its complex roles, VEGFR1 is stimulated by VEGFA. Furthermore, stimulation of VEGFR1 and VEGFR2 is a potential pro-angiogenic therapy [132,133]. Therapeutic angiogenesis shows excellent potential in light of the experimental results. Sadly, no F.D.A.-approved molecule exists [134]. Due to extensive research in this area, the pro-angiogenic molecules are well-categorized. Angiogenic proteins, gene therapy, peptide drugs, and organic molecules are all well-established research areas [135].

Peptides are smaller molecules(around 50 Aa) that, unlike proteins, do not need a tertiary and quaternary structure to be biologically active. Morvore Aa can be easily manipulated and optimized to match the arrangement of Aa present in angiogenesis stimulation molecules. Furthermore, the peptides can be conjugated with organic molecules, or their primary and secondary structures can be modified to fit the desired ADME properties. Given their smaller size and relatively simple structure, the pro-angiogenic peptides can be easily synthesized and released in large amounts to stimulate the angiogenesis pathway (Table 4).

Table 4. VEGF mimetic petides.

N.R.	Name of VEGF mimetic petide	Aa sequence	Reference
1	VEGF-Mimetic Peptide (CBO-P11):	CGGSNH2	[136]
2	VEGF-Mimetic Peptide VEGF-A (86–92)	YKHKGFFQ	[137]
3	VEGF-Mimetic Peptide Vintafolide (EC145)	Ac-SGGR-amino deoxyglucose-folic acid	[138]
4	VEGF-Mimetic Peptide QK-B:	QK-B	[139]
5	VEGF-Mimetic Peptide QK-F11:	QK-F11	[140]
6	VEGF-Mimetic Peptide (YP15):	YP15	[141]
7	VEGF-Mimetic Peptide (AV-3):	EELRYYNKNR	[142]
8	Vascular Endothelial Growth Factor Peptide (VEGF-31):	TNPNRKTKGKE	[143]

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9	VEGF-Mimetic Peptide (ZGDHu-1):	YPDKHLRGD	[144]
10	VEGF-Mimetic Peptide (VGX-1000):	YTRKYKFKIR	[145]
11	VEGF-Mimetic Peptide (LXY30):	LTTSHLLYHLNTKHCFYGG	[146]
12	VEGF-Mimetic Peptide (PRWTEKT)	PRWTEKT	[147]
13	VEGF-Mimetic Peptide (C7):	C7	[148]
14	VEGF-Mimetic Peptide (ZG29)	AGKHLMFGYWKERGRKG	[149]
15	VEGF-Mimetic Peptide (V1):	CTTGRTPR	[150]
16	VEGF-Mimetic Peptide (MF1):	MFYSYFPSD	[151]
17	VEGF-Mimetic Peptide (YLL3):	YLLDVDTKVTP	[152]
18	VEGF-Mimetic Peptide (YLL9)	YLLGLVITGT	[153]
19	VEGF-Mimetic Peptide (RGD-4C)	CRRETAWAC	[154]
20	VEGF-Mimetic Peptide (UPARANT):	AE105-NH2	[155]

Gene therapy for promoting angiogenesis involves introducing genetic material into cells to stimulate the formation of new blood vessels. This approach is explored for various medical conditions, including ischemic diseases, wound healing, and cardiovascular disorders. Here are some examples of pro-angiogenic gene therapies. Some gene therapies are listed in the table below (Table 5):

Table 5. Angiogenesis stimulating molecules.

Nr	Name	Description	Reference
1	Vascular Endothelial Growth Factor (VEGF)	The introduction of the VEGF gene aims to stimulate the production of vascular endothelial growth factor, a key factor in angiogenesis.	[156]
2	Fibroblast Growth Factor (FGF)	FGFs, particularly FGF-2, are involved in angiogenesis. Gene therapy delivering FGF genes can enhance blood vessel formation.	[157]
3	Hypoxia-Inducible Factor-1 (HIF-1)	HIF-1 is a transcription factor that regulates responses to low oxygen levels (hypoxia). HIF-1 gene therapy aims to induce angiogenesis under hypoxic conditions.	[158]
4	Platelet-Derived Growth Factor (PDGF)	PDGF plays a role in cell growth and division, including vascular smooth muscle cells. Gene therapy with PDGF aims to promote vessel formation.	[159]
5	Angiopoietin-1 (Ang-1) Gene Therapy	Ang-1 is involved in stabilizing blood vessels. Gene therapy with Ang-1 aims to enhance vessel maturation and stability	[160]
6	Hepatocyte Growth Factor (H.G.F.)	H.G.F. is known for its angiogenic and tissue regeneration properties. Gene therapy with H.G.F. may promote angiogenesis	[161]
7	hymosin Beta-4 (Tβ4)	Tβ4 is a peptide involved in cell migration, angiogenesis, and tissue repair. Gene therapy with Tβ4 may enhance these processes.	[162]
8	Stromal Cell-Derived Factor-1 (SDF-1)	SDF-1 is involved in recruiting stem cells and promoting angiogenesis. Gene therapy with SDF-1 aims to enhance tissue repair.	[163]
9	Granulocyte-Colony Stimulating Factor (G-CSF)	G-CSF stimulates the production of granulocytes and stem cells and has been explored for its angiogenic potential	[164]

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10 Notch-1 Gene	Notch signaling is involved in vascular development. Gene therapy targeting Notch-1 may influence angiogenesis.	[165]

In the following paragraph, the crucial role in vascular morphogenesis of HIF 1 α Gene is presented.

2.5. HIF-1α Gene

The HIF1α gene, also known as Hypoxia Inducible Factor 1 Subunit Alpha, is crucial in cellular and systemic responses to hypoxia (low oxygen levels). It encodes the alpha subunit of the HIF-1α transcription factor, which forms a heterodimer with a beta subunit. Function: HIF-1α functions as a master regulator by activating the transcription of various genes involved in energy metabolism, angiogenesis (formation of new blood vessels), apoptosis (programmed cell death), and other genes that enhance oxygen delivery or facilitate metabolic adaptation to hypoxia [166]. Importance in Embryonic Vascularization: HIF-1 is essential for blood vessel development during embryogenesis. Tumor angiogenesis: It contributes to the growth of blood vessels in tumors; ischemic disease: HIF-1 α plays a role in the pathophysiology of conditions related to inadequate blood supply, such as ischemic heart disease. H1F-1α variants are alternatively spliced transcript variants encoding different isoforms of H1F1 α have been identified. Its main product is Hypoxia-inducible factor 1-alpha with its crystallographic structure 4h6j [167]. The Aa sequence is the following(Uniprot ID Q16665) HIF1 is a heterodimeric basic helix-loop-helix structure consisting of two subunits: HIF1A (the alpha subunit) and the aryl hydrocarbon receptor nuclear translocator (Arnt, the beta subunit). HIF1A comprises a primary helix-loop-helix domain near the C-terminal, followed by two P.A.S. (PER-ARNT-SIM) domains and a P.A.C. (PAS-associated C-terminal) domain. Additionally, the HIF1A polypeptide contains a nuclear localization signal motif, two transactivating domains (CTAD and NTAD), and an intervening inhibitory domain (I.D.) capable of repressing the transcriptional activities of CTAD and NTAD. Although three HIF1A isoforms are generated by alternative splicing, isoform1 is considered the canonical structure and has been extensively studied in terms of structure and function.

The transcription factor HIF-1 is crucial in mammalian cellular responses to systemic oxygen levels. Its activity, governed by various post-translational modifications like hydroxylation, acetylation, and phosphorylation, regulates multiple genes, including VEGF and erythropoietin. These genes are pivotal in processes such as angiogenesis and erythropoiesis, facilitating increased oxygen delivery to hypoxic areas. HIF-1 also orchestrates the transcription of genes vital for cell proliferation survival and glucose and iron metabolism. In response to fluctuating oxygen levels, HIF-1 undergoes conformational changes and binds to hypoxia-responsive elements (H.R.E.s) on gene promoters, thereby initiating transcription.

Currently, HIF-1α is shown to be implied in proteins involved in cellular responses to changes in oxygen concentration. Extended exposure to hypoxia triggers various cellular mechanisms in skeletal muscles to compensate for limited oxygen levels. One such mechanism involves upregulating specific hypoxia-inducible genes, including vascular endothelial growth factor (VEGF). The VEGF gene is crucial for angiogenesis in skeletal muscles, as evidenced by reduced capillarity upon its deletion in mice. While hypoxia-inducible factor-1alpha (HIF-1alpha) is known to activate VEGF gene transcription by binding to its hypoxic response element (H.R.E.) on the promoter, additional pathways may also contribute to VEGF upregulation during acute or prolonged hypoxia. These pathways, stimulated during hypoxic exposure, may involve inflammation, potentially related to reactive oxygen species generation or changes in cellular energy status indicated by A.M.P. kinase activity. These mechanisms offer different means of VEGF regulation in long-term hypoxic conditions such as those experienced at high altitudes. This review will delve into the cellular signals from hypoxic exposure that could enhance myocyte VEGF expression, including decreased intracellular oxygen levels, skeletal muscle inflammation involving cytokines and oxidative stress, and increased

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A.M.P. kinase activity and adenosine levels associated with reduced cellular energy potential [168]. Some recent findings include pancreatic cancer and hif-1α. Pancreatic cancer, a leading cause of cancer-related deaths worldwide, thrives in a highly hypoxic tumor microenvironment. HIF-1α plays a central role in the carcinogenesis and progression of pancreatic cancer. Researchers have explored how HIF-1α regulates tumorigenesis and progression in pancreatic cancer. Targeting HIF-1α and its signaling pathways could hold promise as a therapeutic approach for pancreatic cancer [169]. Other studies include retinoic acid-induced differentiation; a study investigated HIF-1α's role in retinoic acid-induced differentiation in SH-SY5Y cell cultures under normoxic conditions and low serum concentration. It was found that reduced serum concentrations led to cells remaining in the G0 phase, facilitating cell differentiation [170]. Also, HIF-1α is implied in adipose tissue remodeling. However, the specific mechanisms and implications of HIF-1α in this context are being studied [171]. Also, HIF-1α impacts cancer metabolism, while HIF-1α activation significantly influences cancer cell metabolism. It leads to increased glycolysis and impaired mitochondrial function in tumor cells [172]

While not directly targeting HIF-1α, gene therapy research in P.A.D. aims to improve blood flow and tissue repair. Various approaches, including growth and angiogenic factors, are being investigated in randomized controlled trials [173]. Also, HIF-1α expression and release following endothelial injury are analyzed. Researchers have quantified HIF-1α expression after administration of DMOG (a prolyl hydroxylase inhibitor) in endothelial cells. Understanding HIF-1α dynamics during endothelial injury may provide insights into vascular repair mechanisms [174].

Lastly, in a novel limb ischemia model, intramuscular delivery of AdCA5 (an adenovirus encoding a constitutively active form of HIF-1α) improved perfusion and arterial remodeling. HIF-1α regulates angiogenic growth factors, making it a potential therapeutic target for ischemic conditions. However, current studies do not demonstrate these results that explain the presumably positive benefit as a placebo effect after therapy administration [175–178].

Overall, the latest developments regarding the VEGFR pathway are summarized in the table below (Table 6):

Table 6. VEGFR pathway latest developments.

#	Property	Description	References
1	Targeting Alternative Isoforms	Researchers have been exploring the significance of alternative isoforms of VEGF and VEGFRs and their implications in angiogenesis and cancer. For example, studies have investigated the roles of VEGF-A isoforms (such as VEGF-A165b) and their interactions with VEGFRs in regulating vascular function and tumor progression.	[179]
2	Therapeutic Resistance Mechanisms	There has been increasing interest in understanding the mechanisms underlying resistance to antiangiogenic therapies targeting the VEGF-VEGFR pathway in cancer. Research has focused on elucidating resistance-related molecular pathways, such as activating alternative angiogenic pathways or adaptive changes in tumor cells and the tumor microenvironment.	[180]
3	Development of Novel Therapeutics	Efforts continue to develop novel therapeutic agents targeting the VEGF-VEGFR pathway, including monoclonal antibodies, small molecule inhibitors, and gene therapies. Researchers are exploring combination therapies targeting multiple path components or combining antiangiogenic agents with other treatment modalities to enhance efficacy and overcome resistance.	[181]

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4	Role of VEGFRs in Non-Canonical Signaling	Recent studies have highlighted the involvement of VEGFRs in non-canonical signaling pathways beyond angiogenesis, such as immune modulation, neuroprotection, and metabolic regulation. Understanding these non-angiogenic roles of VEGFRs could lead to novel therapeutic strategies for various diseases.	[182]
5	Emerging Biomarkers	Biomarkers associated with the VEGF-VEGFR pathway are being investigated for their prognostic and predictive value in cancer and other diseases. This includes circulating VEGF levels, VEGFR expression patterns, and genetic variations in VEGF and VEGFR genes, which may help guide treatment decisions and predict patient outcomes.	[183]
6	Role in Neurovascular Diseases	There is growing recognition of the involvement of the VEGF-VEGFR pathway in neuro-vascular diseases, such as stroke, Alzheimer's disease, and diabetic retinopathy. Research is focused on understanding the mechanisms by which VEGF signaling influences neuro-vascular function and exploring its therapeutic potential in these disorders.	[184]
7	Engineering VEGF Mimetics	Scientists are engineering VEGF mimetics and modified VEGF variants with improved pharmacokinetic properties and reduced off-target effects. These engineered ligands aim to enhance therapeutic efficacy and minimize adverse effects associated with VEGF-based therapies.	[185]
8	Role of VEGFRs in Immune Modulation	Recent studies have elucidated the role of VEGF receptors (VEGFRs) in modulating immune responses, particularly in the tumor microenvironment. VEGFR signaling has been shown to influence the expression of inhibitory checkpoints on CD8+ T cells, suggesting a potential crosstalk between angiogenesis and immune regulation in cancer.	[186]
9	Exploring Antiangiogenic Therapies in Combination with Immunotherapy	There is growing interest in exploring the combination of antiangiogenic therapies targeting the VEGF-VEGFR pathway with immunotherapy approaches in cancer treatment. Preclinical and clinical studies have shown promising results, highlighting the potential synergistic effects of targeting both angiogenesis and immune checkpoints in cancer therapy.	[187]
10	Role of VEGF-VEGFR Signaling in Metabolic Regulation:	Recent research has revealed the involvement of VEGF-VEGFR signaling in metabolic regulation beyond angiogenesis. VEGFR signaling has regulated metabolic processes in endothelial cells and other cell types, suggesting potential implications for metabolic diseases and therapeutic interventions.	[188]
11	Therapeutic Targeting of VEGF-VEGFR Pathway in Neurodegenerative Diseases	The VEGF-VEGFR pathway has emerged as a potential therapeutic target in neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease. Studies have highlighted the neuroprotective effects of VEGF signaling and its potential implications for	[189]

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		disease-modifying therapies in neurodegenerative disorders.	
12	Role of VEGF-VEGFR Signaling in Organ Development and Regeneration:	Research has uncovered the crucial role of VEGF-VEGFR signaling in organ development and regeneration processes. Endothelial-derived endocrine signals mediated by VEGFR signaling have been shown to induce and sustain regenerative processes in various organs, suggesting therapeutic potential for tissue engineering and regenerative medicine.	[190]
13	Mechanisms of VEGF-VEGFR Axis in Cancer Metastasis:	Recent studies have provided insights into the mechanisms underlying the involvement of the VEGF-VEGFR pathway in cancer metastasis. VEGF-VEGFR signaling has promoted tumor cell dissemination and metastatic spread through various mechanisms, including angiogenesis-independent effects on tumor cells and the tumor microenvironment.	[191]
14	Exploring VEGF-VEGFR Signaling in Tissue Engineering and Regenerative Medicine:	The VEGF-VEGFR pathway has been explored in tissue engineering and regenerative medicine to promote vascularization and tissue regeneration. Studies have investigated using VEGF-based therapies and engineered constructs to enhance vascularization and improve the functional outcomes of tissue engineering approaches.	[192]
15	Role of VEGF-VEGFR Pathway in Age-Related Macular Degeneration (AMD	The VEGF-VEGFR pathway plays a crucial role in the pathogenesis of age-related macular degeneration (AMD), a leading cause of vision loss in the elderly. Anti-VEGF therapies targeting this pathway have revolutionized the treatment of AMD by inhibiting pathological neovascularization and preserving vision.	[193]

In the next paragraph, cell chirality in the light of the vasculogenesis process is presented.

2.6. Cell Chirality Vasculogenesis

The majority of macromolecules present in cells exhibit chirality, indicating that they cannot be overlaid onto their mirror images. Nonetheless, cells can exhibit chirality, a topic that has garnered minimal attention until recently.

Consequently, chirality at the cellular level may significantly influence left-right asymmetric development in several invertebrate species. Recent reports indicate that cell chirality has been observed in numerous cultured vertebrate cells, with research suggesting that this phenomenon is evolutionarily conserved, highlighting the critical function of the actin cytoskeleton. The biological functions of cell chirality in vertebrates are still to be elucidated. However, it may regulate left-right asymmetric development or other morphogenetic processes. The exploration of cellular chirality has recently commenced, and this emerging discipline is expected to yield significant insights in biology and medicine.

The helical structure of cells is recognized for its essential function in development and illness. Nonetheless, the fundamental mechanism driving this behavior remains predominantly unexamined, especially in replicating it within rigorously regulated engineering systems. Utilizing sophisticated microfluidics it provided substantial evidence of the spontaneous formation of helical endothelial tubes demonstrating strong right-handedness dictated by intrinsic cell chirality. Modulating endothelial cell chirality with small-molecule pharmaceuticals induces a dose-dependent inversion of handedness in constructed arteries alongside non-monotonic alterations in vascular permeability.

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The morphogenesis of tubular tissues or organs is a crucial process that takes place during early development in various animals. Structures like the embryonic heart tubes in vertebrates and the embryonic hindgut in Drosophila are among the initial instances of left-right (LR) symmetry breakdown. Prior research has shown that left-right asymmetry during tubular morphogenesis might arise from cellular chirality. The inherent left-right asymmetric feature of cells is involved in diverse processes across different morphological scales, with the cellular chiral bias showing significant alignment with the handedness observed at the multicellular or tissue level. Despite significant advancements in elucidating the significance of cell chirality in the tubular morphogenesis of epithelial and cardiac tissues, the understanding of left-right asymmetry in typical endothelial blood vessels, another common tubular form, remains considerably constrained.

Furthermore, in the natural environment, blood arteries are predominantly lined with endothelial cells on the inner surface of the cylinder wall, with their apical side oriented towards the vessel's center. These cells provide a compact endothelial lumen, delineating the intravascular milieu from the extravascular space and facilitating the dynamic modulation of vascular permeability. It was previously observed that endothelial cells have a pronounced clockwise (CW) chiral bias. The neutralization or randomization of this CW bias led to junctional disruptions and increased endothelial permeability due to the failure to establish intact connections between cells exhibiting heterogeneous chirality. Although the regulatory roles of cell chirality in endothelial functions are well-established, contemporary research on the morphological and biophysical characteristics of tubular vessels has predominantly concentrated on cell behaviors or cues that are longitudinal, circumferential, or perpendicular to the curved substrate surface, neglecting the implications of cell handedness or related biases in cell alignments and mechanical forces.

Also, the demonstration of LR asymmetry or chirality in a system generally necessitates two established axes, namely the apical-basal (AB) axis and the front-rear (FR) axis. Nonetheless, the polarization of the tissue along the longitudinal axis is not consistently evident, as observed in a growing heart when no directional guidance is present. Blood flow is directed. It is missing during the earliest phase of vascular formation and angiogenesis, therefore failing to elucidate cell polarization completely. This prompts an inquiry about the manifestation of the chiral characteristic of endothelial cells morphologically on the tubular substrate, given the presence of just the AB axis. While blood arteries do not experience tissue or organ-scale asymmetrical morphogenesis akin to the chiral C-looping of heart tubes, numerous investigations utilizing in vitro three-dimensional (3D) vascular platforms demonstrate a helical alignment of endothelial cells along tubular geometries. This issue has not been thoroughly examined, and handedness has not been objectively evaluated.

Endothelial cells can autonomously develop a right-handed helical structure in both in vitro tubular substrates and in vivo vascular tissues. The reversal of inherent cellular chirality modified the helical handedness of the vasculature, corroborated by in vitro tests, including PKC activation and computer simulations of chiral torque force direction switching.

In vitro models have been extensively utilized in biomedical research. Over the past century, Petri plates have served as a fundamental model for cell culture, yielding significant insights into the biophysical and metabolic characteristics of cells within live organisms. Transwell membranes facilitate epithelial cell culture by establishing the appropriate apicobasal polarity observed in vivo, marked by pronounced actin and atypical protein kinase C expression at the apical surface, with the deposition of basement extracellular matrix at the basal surface. The in vitro scratch experiment, which involves creating a linear gap in a cellular monolayer, facilitates the investigation of cell migration during wound healing and the analysis of front-rear polarity. These approaches have facilitated considerable research on apicobasal and front-rear polarity in many pieces of literature, resulting in a thorough grasp of molecular pathways thanks to these straightforward in vitro models. The polarity along the left-right (LR) axis has not been extensively investigated at the cellular level in a controlled manner until the recent emergence of various in vitro cell chirality systems.

The formation of the LR axis is essential for living creatures. All vertebrates demonstrate asymmetry along the body's midline, referred to as handedness or chirality, in the arrangement and structure of internal organs. A variation from this arrangement frequently results in significant

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implications, particularly when one or two organs are positioned in a mirrored orientation (i.e., situs ambiguus). In cases of situs inversus, where all internal organs are transposed to the opposite side of the body, individuals may remain healthy; nevertheless, some may experience conditions such as Kartagener syndrome. Various models have been developed to elucidate the mechanisms of symmetry breaking in animal embryos, focusing on critical factors such as the leftward fluid flow at the ventral node induced by ciliary rotation, voltage gradients arising from the asymmetric expression of ion channels, and asymmetric vesicular transport mediated by unconventional myosin ID along actin cable network. Laterality defects are present in more than 0.1% of live births. The actual percentage may be higher, as inadequate axis establishment in fetuses frequently results in miscarriages. Consequently, investigating the establishment of the LR axis holds considerable clinical significance.

Growing data indicates that cell chirality may play a crucial role in the left-right asymmetry of embryonic development. An object (e.g., D-glucose and DNA) is deemed chiral when it can be differentiated from its mirror image. Cell chirality mathematically refers to the relationship of handedness (left or right) between the LR axis and the established apicobasal axis (often arising from two-dimensional cell attachment) and the front-rear axis (generally determined by the nuclear-centrosomal axis in polarized cells). Chirality can also be recognized as the directional rotation of cellular organelles, the cytoskeleton, and whole cells. Due to the inherent randomness in the morphology and movement of individual cells, cell chirality is typically characterized as a statistical property of populations at subcellular, cellular, and multicellular levels. It is quantified through the directional biases in organelle positioning, cytoskeletal dynamics, cell shape, alignment, and migration. Cell chirality has been documented in various biological systems throughout developmental biology. Xenopus embryos and parthenogenetically activated eggs, when cleaved and treated with the myosin ATPase inhibitor 2,3-butanedione monoxime, demonstrated significant chiral twisting of actin structures. The chirality of planar cell shape was identified as the determinant of directional looping in the hindgut of Drosophila. Comparable outcomes were seen for the rotation of Drosophila genitalia. Formin, an essential scaffolding protein linked to cellular chirality, was identified as being associated with left-right asymmetry in the pond snail and frog.

Research on LR asymmetry has been gained from in vitro models of cellular chirality. Initially, in vitro models integrate minimal stimuli by omitting potential confounding variables, hence significantly enhancing the elucidation of molecular pathways. Secondly, akin to Petri plates, in vitro systems are generally user-friendly, allowing for reliable imaging and molecular tests. Third, these approaches may be more effective in screening teratogens and environmental variables linked to laterality-related birth abnormalities. Ultimately, utilizing human cells, in vitro investigations may elucidate patient-specific pathways linked to deformities (Table 7).

Table 7. Chirality in Endothelial Cells.

#	*Factor*	*Description*	*Ref*
1	Cellular Architecture:	The intrinsic asymmetry of the cytoskeleton (e.g., actin filaments and microtubules) contributes to the chirality of endothelial cells, influencing their shape and movement.	[194]
2	Extracellular Matrix (ECM) Composition:	The composition and organization of the ECM can provide directional cues, affecting cell alignment and orientation. ECM components like collagen and fibronectin can promote specific cellular behaviors.	[195]
3	Mechanical Forces:	Shear stress from blood flow exerts mechanical forces on endothelial cells, which can influence their orientation and organization. Cells respond to these forces in a way that can enhance their chiral characteristics.	[196]
4	Cell-Cell Interactions:	Adhesion molecules (e.g., cadherins, integrins) facilitate interactions between endothelial cells. The arrangement of these	[197]

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		molecules can affect how cells align and respond to external signals.	
5	Growth Factors:	Signals from growth factors (e.g., VEGF, FGF) can promote angiogenesis and influence the spatial arrangement of endothelial cells. These factors can also affect cellular chirality by promoting asymmetric growth or proliferation.	[198]
6	Gene Expression:	Differential gene expression can lead to asymmetries in protein distribution within endothelial cells, impacting their chirality. Genes involved in cytoskeletal dynamics and cell signaling are critical.	[199]
7	Cell Polarity:	Endothelial cells exhibit intrinsic polarity, which is critical for their function. Polarity can influence the orientation of cellular processes and the distribution of organelles, contributing to chirality.	[200]
8	Environmental Cues:	Factors such as hypoxia or inflammation can alter endothelial cell behavior, potentially affecting their chirality by modifying how they interact with each other and with their environment.	[201]
9	Tissue Context:	The surrounding tissue environment can influence endothelial cell behavior. For instance, the architecture and mechanical properties of neighboring tissues can provide context for chirality.	[202]
10	Pathological Conditions:	Conditions such as atherosclerosis or cancer can disrupt normal endothelial cell chirality, leading to aberrant vascular structures and functions.	[203]
11	Cytoplasmic Streaming:	The movement of cytoplasmic contents can affect the distribution of organelles and signaling molecules, contributing to cellular asymmetry.	[204]
12	Cell Cycle Dynamics:	The stage of the cell cycle can influence cellular behavior and polarization, impacting chirality during processes like division and migration.	[205]
13	Signaling Pathways:	Pathways such as Wnt, Notch, and Hippo play roles in regulating cell fate and polarity, potentially affecting the chirality of endothelial cells.	[206]
14	Topography of the Substrate:	The physical properties and microstructure of the substrate on which endothelial cells grow can influence their alignment and behavior, promoting chirality.	[207]
15	Cell Density:	The density of endothelial cells can impact how they interact with one another and their ECM, influencing collective behavior and orientation.	[208]
16	Vascular Fluid Dynamics:	Changes in blood flow patterns (e.g., turbulence vs. laminar flow) can affect endothelial cell alignment and shape, impacting their chirality.	[209]
17	Matrix Stiffness:	The mechanical properties of the ECM, including stiffness, can influence cell behavior and differentiation, contributing to asymmetric structures.	[210]
18	Inflammatory Signals:	Cytokines and chemokines released during inflammation can alter endothelial cell behavior, leading to changes in their chirality and organization.	[211]
19	Cellular Mechanotransduction:	The ability of cells to sense and respond to mechanical stimuli can influence their morphology and alignment, thereby affecting chirality.	[212]

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20	Genetic and Epigenetic Factors:	Genetic variations and epigenetic modifications can lead to differences in how endothelial cells express chirality-related genes, impacting their behavior.	[213]
21	MicroRNA Regulation:	MicroRNAs can regulate the expression of genes involved in cell polarity and cytoskeletal dynamics, influencing the chirality of endothelial cells.	[214]
22	Cell-Extracellular Matrix Interactions:	Specific interactions between endothelial cells and ECM components can create asymmetric signals that contribute to cellular chirality.	[215]
23	Intercellular Communication:	Signaling through gap junctions or extracellular vesicles can promote coordinated behavior among endothelial cells, influencing their collective chirality.	[216]
24	Hormonal Regulation:	Hormones can modulate endothelial cell functions, including their response to shear stress and their migratory behavior, which can impact chirality.	[217]
25	Biochemical Gradients:	The presence of biochemical gradients in the environment can direct cell migration and organization, leading to asymmetric distributions.	[218]

The cellular chirality indexes that play a role in vasculum genesis are discussed in the following paragraph.

2.7. Cellular Chirality Indices

Cellular chirality indices are quantitative metrics employed to characterize and quantify the chirality (or handedness) in the structure or activity of cells. Cellular chirality is a characteristic observed in multiple biological situations, ranging from tissue-level asymmetries to the morphologies and behaviors of individual cells, including cell migration. Measuring chirality aids researchers in comprehending the emergence of left-right asymmetry in biology, influencing areas such as developmental biology, tissue engineering, and disease modeling (Table 8).

Table 8. Cellular Chirality Indices.

Nr	DESCRIPTOR	DESCRIPTION	REFERENCES
1	Cell Orientation Index (COI)	This index measures the angular orientation of cells with respect to a reference direction. COI is calculated based on the alignment of cellular components or the elongation axis of the cell. For chiral cells, the orientation may consistently deviate to one side, which can be quantified.	[219]
2	Cell Shape Index (CSI)	CSI measures the asymmetry in the cell shape, precisely capturing how cell boundaries deviate in a preferred direction, indicating chirality. Typical metrics include the aspect ratio and elongation direction, which are then analyzed for directional bias.	[220]
3	Cell Movement Chirality Index (CMCI):	For migratory cells, the CMCI captures the directionality in their movement. This index considers the trajectory of cell movement over time, focusing on whether cells exhibit a consistent rotational or directional bias (clockwise or counterclockwise).	[221]
4	Left-Right Asymmetry Index (LRAI):	LRAI quantifies asymmetry in specific cellular structures that may display handedness, such as the cytoskeleton or membrane proteins. This is often done by identifying and	[222]

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		analyzing the spatial distribution of specific biomolecules in the cell.	
5	Vorticity and Angular Velocity Indices:	These indices measure rotational behaviors, such as the angular velocity of cell movement or rotational flow patterns in tissue. Angular velocity is particularly relevant in tissues with chiral rotational patterns, like embryonic development or organ formation.	[223]
6	Molecular ChiralityIndex (MCI):	This index analyzes the orientation of chiral molecules within cells, such as actin or microtubule structures, that may display helical or asymmetrical patterns. MCI helps identify molecular basis contributions to overall cellular chirality.	[224]
7	Skewness in Fluorescence Signal Distribution:	For cells with fluorescently tagged proteins or structures, skewness in the spatial distribution of fluorescence intensity can serve as a measure of chirality. This technique is often used to identify asymmetry in protein localization or membrane curvature.	[225]
8	Nuclear Rotational Chirality Index	This index focuses on nuclear rotation during cell spreading on a substrate, measuring whether cells exhibit a clockwise (CW) or counterclockwise (ACW) rotational bias. Studies on fibroblast cells, for example, have shown a shift from CW to ACW rotation depending on cell morphology and spread area, offering insights into cytoskeletal reorganization during attachment.	[226]
9	Multi-cellular Swirling Chirality:	Cells with individual chirality can form coherent swirling patterns at the tissue level. Boundary constraints and cell chirality coordination regulate this, which is relevant to understanding tissue-level asymmetry in developmental biology. Multi-cellular swirling is critical for studying coordinated chirality in cell groups, especially on patterned substrates.	[227]
10	Asymmetric Cell Division Index	Cell chirality often influences asymmetric cell division, where the spatial orientation of the mitotic spindle correlates with handedness. This index tracks deviations in spindle orientation and can explain how chirality at the cellular level might impact tissue patterning and developmental asymmetry (referenced in cardiovascular development studies)	[228]
11	Cytoskeletal Filament Bias Index	This index quantifies the structural bias in cytoskeletal filaments like actin or microtubules. During cardiovascular development, for instance, the chiral bias in filament orientation helps guide asymmetrical tissue formation, such as cardiac looping. As observed in heart and vessel development, this index can reveal crucial links between cellular structure and organ asymmetry.	[229]
12	Polarity Vector Index	This index measures the orientation of the cell's polarity axis relative to a reference direction. By examining the distribution of polarization vectors (based on protein markers, such as PAR proteins), researchers can determine if there is a directional bias that indicates chirality. This	[230]

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		index is essential in cell migration and orientation studies, particularly in asymmetric tissue formation (	
13	Directional Migration Index (DMI)	Used to quantify the preferred migration direction in cells, DMI analyzes whether cells move consistently to one side, a characteristic of chiral migration. This index is handy in cancer metastasis studies and tissue morphogenesis, where a chiral bias can influence how cells interact with their environment and migrate collectively.	[231]
14	Organellar Localization Chirality Index:	Measures the asymmetry in the spatial positioning of intracellular organelles, such as the Golgi apparatus or nucleus. This chirality is often observed in asymmetrically dividing cells and in developmentally significant cell types, where biased organelle positioning guides cellular polarization and division direction.	[232]
15	Extracellular Matrix (ECM) Alignment Index	Focuses on the alignment of ECM fibers relative to cell orientation. Many cells exhibit chirality by aligning ECM fibers in a chiral manner, impacting cell signaling and mechanical cues. This index is particularly relevant in tissue engineering and wound healing, where ECM organization is critical for functional tissue formation	[233]
16	Membrane Curvature Chirality Index:	This measures the curvature direction of cell membranes, especially important in cells with polarized shapes, like neurons. Membrane curvature is often chiral, impacting cellular processes such as endocytosis and signaling. This index is used in neuroscience and cell signaling studies, revealing how membrane chirality affects cell function	[234]
17	Cell Alignment and Collective Rotation Index:	Tracks the alignment and rotational direction of cell groups. In many tissues, collective cell rotation exhibits a chiral preference, such as in epithelial sheet migration and certain cancer cell clusters. This index is useful in studying coordinated cell migration and the mechanics of tissue formation	[235]
18	Microtubule Organizing Center (MTOC) Positioning Index	This index measures the positional bias of the MTOC within the cell, which can reflect chiral intracellular organization. MTOC positioning plays a key role in establishing cellular polarity and directional migration, particularly in immune cells and migrating fibroblasts	[236]
19	Golgi Apparatus Orientation Index	This index examines the positioning of the Golgi apparatus relative to the nucleus and other cell structures. Chiral bias in Golgi orientation is important in cell migration and polarity, as the Golgi often faces the leading edge in polarized cells. This index is helpful in studying directional cell behavior in contexts like embryonic development and cancer cell invasion	[237]
20	Apical-Basal Chirality Index in Epithelial Cells:	Epithelial cells often exhibit chirality in the orientation of their apical and basal surfaces, affecting how they align and form tissue structures. This index measures any consistent bias in apical-basal polarity across cells, crucial for understanding tissue morphogenesis in organs like the gut and heart	[238]
21	Focal Adhesion Rotation Index	This index tracks the rotational direction and alignment of focal adhesions within a cell, which are often arranged	[239]

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		with a directional bias in cells with chiral behavior. Focal adhesion rotation has been associated with directional migration and extracellular matrix remodeling, particularly relevant in wound healing and cancer research	
22	Mitochondrial Distribution Chirality Index	This examines the asymmetrical positioning of mitochondria within cells. Mitochondria often localize with a chiral bias around the cell's cytoskeleton, which influences energy distribution and intracellular signaling. This index is critical in studies on metabolic diseases and polarized cells	[240]
23	Chiral Stress Fiber Alignment Index	Stress fibers, composed of actin filaments, can exhibit chiral alignment that supports cellular contractility and polarization. This index measures the orientation of these fibers within the cell and their rotational alignment. It is valuable in exploring cellular mechanics in muscle cells and other contractile cell types	[241]
24	Nuclear Envelope Asymmetry Index	Some cells exhibit chiral bias in the shape or positioning of the nuclear envelope, impacting gene expression and signaling pathways. This index captures deviations from symmetry in nuclear shape and organization, particularly important in developmental biology and diseases involving nuclear envelope abnormalities.	[242]
25	Cell Boundary Curvature Chirality Index	Measures curvature along cell boundaries, which can indicate chirality, especially in epithelial cells during development. This index is often used in morphogenesis studies where cell shapes are asymmetrically patterned.	[243]
26	Actin Spiral Index	This index measures the extent and direction of actin filament spiraling within cells, often related to cell motility and migration chirality.	[244]
27	Myosin II Orientation Index	Measures the orientation and alignment of Myosin II filaments, which are critical for asymmetric contractile forces during chiral cell behaviors.	[245]
28	Golgi Apparatus Polarization Index	Focuses on the spatial bias of the Golgi apparatus within polarized cells. Asymmetrical Golgi positioning can contribute to chiral orientation in many cell types.	[246]
29	Endocytosis Orientation Index	Measures directional bias in endocytosis events within cells, which can exhibit chirality in cell types such as neurons and epithelial cells.	[247]
30	Cell Protrusion Chirality Index	Quantifies the chiral orientation and bias of cellular protrusions like filopodia or lamellipodia, critical in migratory cells.	[248]
31	Mitochondrial Asymmetry Index	It measures the asymmetrical distribution of mitochondria within cells, which is relevant for understanding chiral orientation in polarized cell types.	[249]
32	Lipid Raft Distribution Chirality Index	This index focuses on the spatial asymmetry of lipid rafts in the plasma membrane, which often show directional bias influencing signaling pathways.	[250]
33	Cilium Rotation Chirality Index	Used to measure the rotational bias of cilia, which is essential for directional fluid flow and cellular signaling, particularly in respiratory and reproductive systems.	[251]

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34	Axonal Growth Chirality Index	Quantifies directional bias in axonal extension, particularly relevant in neurodevelopment where axons show chiral growth patterns.	[252]
35	Glycocalyx Asymmetry Index	Measures the distribution of glycocalyx components on cell surfaces, which often exhibit chiral organization and are essential for cellular interaction and recognition.	[253]
36	Protein Localization Bias Index	It focuses on the chiral localization of specific proteins within cells, which can impact polarity and signaling in asymmetric cell divisions and migrations.	[254]
37	Microvilli Alignment Chirality Index	This index evaluates the directional orientation of microvilli, which are often arranged asymmetrically to maximize absorptive efficiency, particularly in epithelial cells.	[255]
38	Endoplasmic Reticulum (ER) Distribution Index	This measures asymmetry in ER structure, as the ER's orientation is often polarized to influence protein synthesis and trafficking within cells.	[256]
39	Cell Surface Receptor Chirality Index	Measures the asymmetric distribution of specific receptors on the cell surface, which may affect signaling and interactions with the environment.	[257]
40	Actin Polymerization Rate Index	Focuses on the rate and direction of actin polymerization in response to chiral cues, which plays a role in cell motility and shape.	[258]
41	Nuclear Shape Chirality Index	Assesses nuclear shape asymmetry, which may reflect underlying cellular polarity and affects gene expression and cellular response to mechanical stress.	[259]
42	Mitochondrial Fusion/Fission Chirality Index	Quantifies the chiral patterns in mitochondrial fusion and fission processes, which are critical for cellular energy balance and metabolic function.	[260]
43	Lysosomal Positioning Chirality Index	Tracks the asymmetric positioning of lysosomes within cells, which can influence cell polarity and affect intracellular degradation pathways.	[261]
44	Cell-Surface Glycosylation Chirality Index	Measures the chiral arrangement of glycosylated molecules on the cell surface, important for cell recognition and immune interactions.	[262]
45	Rho GTPase Activity Chirality Index	Focuses on the asymmetric activation of Rho GTPases, which are crucial in regulating cell polarity, shape, and migration.	[263]
48	Endoplasmic Reticulum-Golgi Orientation Index	Measures the spatial relationship between the ER and Golgi, which can have a chiral influence on intracellular trafficking and cell polarity.	[264]
49	Apoptotic Body Formation Chirality Index	Quantifies the chiral orientation of apoptotic bodies during programmed cell death, which can influence tissue organization and immune response.	[265]
50	Nucleolar Positioning Chirality Index	This measures the chiral bias in nucleolar positioning within the nucleus, which may impact gene expression and cell cycle regulation.	[266]

Furthermore, a generalized view is required, so in the next paragraph, the tissue chirality indices are presented.

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2.8. Tissue Indices

Tissue indices are quantitative metrics employed to assess the organization, structure, and functionality of cellular populations within tissues. In contrast to cellular indices, which emphasize individual cells, tissue indices reflect traits that arise from the aggregate behavior and organization of several cells. These indices assist researchers in comprehending the collaborative functioning of cells to create cohesive, functional structures like organs, and they are frequently employed in investigations of tissue formation, morphogenesis, wound healing, and disease progression (Table 9).

Table 9. Tissue Indicies.

Nr.	DESCRIPTOR	DESCRIPTION	REF
1	Tissue Polarity Index	Quantifies the directional alignment of cells within a tissue layer, which is crucial for forming ordered structures and for processes like epithelial morphogenesis.	[267]
2	Epithelial Sheet Rotation Index	Measures the coordinated rotational movement of epithelial cells, often observed in wound healing and during developmental tissue movements.	[268]
3	Multi-cellular Swirling Chirality Index	Captures the collective swirling or chiral rotation of cell clusters, a behavior observed in developmental processes and certain tissue cultures.	[269]
4	Tissue Rigidity Index	Quantifies the stiffness or elasticity of a tissue, which influences cell behavior, particularly in cancer research, where increased stiffness is often associated with tumor progression.	[270]
5	Cell Density Index	Measures the number of cells within a given tissue area, used to assess tissue growth, cell proliferation, or cell death rates.	[271]
6	Tissue Curvature Index	Quantifies the curvature or bending of tissues, especially relevant in understanding how organs like the brain or intestines develop their complex shapes.	[292]
7	Vascularization Index	It measures the density and distribution of blood vessels within a tissue, which is crucial for assessing nutrient supply, oxygenation, and overall tissue health.	[273]
8	Extracellular Matrix (ECM) Density Index	Measures the density and organization of ECM proteins (e.g., collagen, fibronectin) within a tissue. This index is crucial for understanding how the ECM supports cellular adhesion, migration, and tissue stiffness.	[274]
9	Collagen Fiber Orientation Index	Evaluates the directional alignment of collagen fibers within tissues, which can impact tissue strength, elasticity, and directional cell migration.	[275]
10	Inflammatory Cell Density Index	Quantifies the presence of immune cells within a tissue, often used to assess inflammation, immune response, and tissue repair.	[276]
11	Fibrosis Index	Measures the extent of fibrotic tissue (scar tissue) formation, usually by assessing collagen deposition and organization, important in studying chronic disease and tissue repair.	[277]
12	Oxygenation Index	Quantifies tissue oxygen levels, which are critical in studying tissue viability, function, and the development of hypoxic regions, especially in tumors and ischemic tissues.	[278]
13	Adiposity Index	Measures the amount of adipose (fat) tissue within an organ or area, commonly used in studies of obesity, metabolic disorders, and certain cancers.	[279]

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14	Cell Proliferation Index	Assesses the rate of cell division within a tissue, often using markers like Ki-67 to evaluate tissue growth, regeneration, or cancer cell proliferation.	[280]
15	Apoptosis Index	Measures the rate of programmed cell death in tissues, often through TUNEL staining, which is essential for understanding tissue homeostasis and responses to damage or disease.	[281]
16	Angiogenesis Index	Quantifies new blood vessel formation within a tissue, often used to assess tumor growth, wound healing, and cardiovascular disease.	[282]
17	Neurogenesis Index	Measures the generation of new neurons in brain tissue, often used in studies of brain development, learning, and neurodegenerative disease.	[283]
18	Myelination Index	Measures the extent of myelin covering axons within nervous tissue, which is vital in understanding brain development, multiple sclerosis, and other neurological conditions.	[284]

Furthermore, the differences between cellular and tissue indices are discussed.

2.9. Cellular Indices vs. Tissue Indices

Cellular indices quantify specific features, structures, or behaviors within individual cells. They focus on parameters like cell shape, polarity, migration patterns, organelle positioning, and molecular organization within single cells. These indices help researchers study cell-level processes like cellular chirality, migration, polarity, and the effects of intracellular components on overall cell function. Cellular indices are commonly used in research on cancer metastasis, immune response, and cell differentiation. Nuclear Rotational Chirality Index, Actin Polymerization Rate Index, and Golgi Apparatus Orientation Index, all of which capture unique aspects of cellular orientation and asymmetry relevant to single-cell behaviors.

Tissular indices measure properties that emerge from the collective organization, interaction, and alignment of multiple cells within a tissue. These indices capture the behavior of cells as a cohesive unit within a tissue matrix, focusing on the structural and functional arrangement that arises when cells work together. Tissular indices are important for understanding processes like tissue morphogenesis, organ development, and wound healing, where the behavior of cell groups (rather than isolated cells) drives biological outcomes. They are also valuable in studying tissue-level chirality, such as the rotation of cell layers in developing organs. Epithelial Sheet Rotation Index, which tracks coordinated rotation in epithelial cells; Multi-cellular Swirling Chirality, used to study how groups of cells orient themselves collectively on substrates; and Tissue Polarity Index, which assesses overall alignment within tissue sections.

Cellular indices focus on intracellular and single-cell processes, while tissular indices evaluate intercellular and collective tissue dynamics. Cellular indices assess local factors (within or affecting one cell), whereas tissular indices evaluate the organization and behavior of cell populations, capturing emergent properties that are not observable at the single-cell level. Cellular indices are more relevant in studies involving isolated cells in culture, whereas tissular indices are essential in vivo studies of organ development and functional tissue organization.

Together, these two categories of indices provide a full spectrum for understanding how both individual cell properties and collective cell behaviors contribute to the structural and functional organization of tissues and organs (Table 10).

Table 10. Cellular Indices Vs. Tissue Indices.

Nr	PARAMETER	CELLULAR INDICES	TISSUE INDICES	REF
1	Scale of Measurement	Focus on individual cells and their components, measuring properties such as cell shape, migration,	Evaluate collective properties and interactions across cell populations, capturing the	[285]

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		polarity, and intracellular processes (e.g., actin alignment, nuclear rotation). They capture detailed characteristics at a single-cell level, offering insight into individual cell behaviors.	emergent organization and structure in tissues. Tissue indices consider properties like tissue stiffness, alignment, vascular density, and cellular arrangement in a way that reflects collective cell dynamics in a multicellular environment.	
2	Complexity and Emergent Properties	Generally simpler, focusing on the direct measurement of a specific trait within individual cells. They are highly useful for identifying cellular responses to microenvironmental factors or genetic changes and are foundational in single-cell studies.	Capture complex, emergent properties that arise only when cells work collectively within a tissue, such as tissue rigidity, angiogenesis, and multi-cellular chirality. These properties cannot be observed at the single-cell level and require interaction across multiple cells and extracellular matrix components to manifest.	[286]
3	Functional and Structural Focus	Primarily used to understand intracellular functions and mechanisms, such as gene expression, cellular signaling, and cell motility, or structural features like organelle orientation and polarity.	Assess structural and functional properties of tissues, such as tissue organization, collective cell alignment, and spatial arrangement, and examine how these impact tissue-level functions like nutrient transport, mechanical strength, and coordinated growth.	[287]
4	Research Applications and Relevance	Vital in fields like cell biology, pharmacology, and molecular biology, especially in applications such as studying drug effects on cellular pathways, understanding single-cell motility, and examining cell response to microenvironmental changes.	It is more commonly used in developmental biology, oncology, tissue engineering, and regenerative medicine, where collective cell behaviors and structural organization influence outcomes like tumor formation, wound healing, and tissue regeneration.	[288]
5	Analytical Techniques and Tools	Often analyzed using microscopy techniques (e.g., fluorescence microscopy, live-cell imaging) and molecular assays that target specific cellular components or pathways, like Western blotting or PCR for particular markers.	Require more complex imaging methods, like histology, tissue staining (e.g., Masson's Trichrome for fibrosis), and MRI, as well as biomechanical measurements (e.g., tensile testing) and computational models to assess mechanical properties and structural organization.	[289]

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6	Sensitivity to Microenvironment	More sensitive to immediate microenvironmental factors like substrate stiffness, chemical gradients, and local signaling molecules, which directly impact cell morphology, migration, and other behaviors.	Reflect larger-scale environmental interactions, like oxygenation, nutrient supply, and the presence of immune cells, which influence tissue health, adaptation, and pathology, particularly in cases like chronic inflammation, tumor growth, and ischemia.	[290]
7	Temporal Dynamics	It can capture rapid changes in individual cell behavior and response to stimuli in real-time or short-term studies, which is ideal for dynamic cellular processes like migration and division.	Often track slower, long-term changes in tissue structure and organization, making them suitable for studying developmental processes, chronic disease progression, and tissue remodeling.	[291]
8	Degree of Interaction	Typically measure isolated cell behaviors with a focus on intracellular interactions (e.g., cytoskeletal dynamics, organelle positioning). These indices don't often capture intercellular interactions except in cases where cellular contact is required.	Reflect interactions between multiple cells and between cells and the extracellular matrix (ECM), which create structural properties like tissue rigidity and polarity. This higher-order complexity is essential for understanding phenomena like coordinated cell migration and tissue remodeling.	[292]
9	Spatial Orientation and Dimensionality	Often measured in two-dimensional (2D) cultures where individual cells are analyzed in isolation. They are limited in capturing three-dimensional (3D) spatial orientation, which is more relevant in native tissue environments.	Measured in three-dimensional contexts that more closely mimic in vivo conditions, capturing 3D organization and alignment, such as in 3D tissue scaffolds or organoids. This spatial complexity is crucial for studying properties like tissue polarity and curvature.	[293]
10	Measurement Focus	Focus on physiological properties relevant to cell-specific functions, such as cellular metabolism, apoptosis, or mitosis rates, which reveal the health and functional status of individual cells.	Capture tissue-wide functional metrics like vascularization, oxygenation, and tissue stiffness, focusing on properties that maintain overall tissue viability and function.	[294]
11	Temporal Stability and Dynamics	Often capture rapid, short-term responses, such as signaling cascades, cellular contraction, or migration speed, ideal for studying acute responses to stimuli.	Can assess long-term, more stable properties like ECM remodeling, tissue stiffening, and fibrosis development, providing insights into chronic changes like aging and disease progression.	[295]

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12	Response to Mechanical Forces	Measure how individual cells respond to local mechanical forces, often on a microscale, such as cell stretching, compression, or substrate stiffness. These forces are directly sensed by cells via mechanoreceptors.	Reflect the mechanical properties of an entire tissue, such as elasticity, compressive strength, and tensile strength, which arise from collective cell behavior and ECM interactions, influencing tissue integrity and resilience.	[296]
13	Data Collection and Quantification Complexity	Collected through relatively straightforward methods like fluorescence microscopy, flow cytometry, and live-cell imaging, which focus on specific cellular markers or behaviors.	Often require advanced imaging techniques such as magnetic resonance imaging (MRI), confocal microscopy, or biomechanical testing, allowing for detailed analysis of spatial and structural organization within tissue sections.	[297]
14	Biological and Pathological Relevance	Useful for understanding specific cellular functions and dysfunctions, such as in drug testing, toxicity studies, or targeted gene editing, where single-cell responses are analyzed.	Provide insights into complex, multicellular responses, such as inflammation, fibrosis, or cancer metastasis, where tissue-wide coordination and environmental cues are essential.	[298]

The VEGF-VEGFR pathway and implications in cellular chirality are further discussed.

2.10. VEGF -VEGFR Signaling and Cellular Chirality

Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) play critical roles in cellular processes like angiogenesis (formation of new blood vessels), which is essential for supplying tissues with oxygen and nutrients. Research is increasingly revealing connections between VEGF/VEGFR signaling and cellular chirality, particularly in the context of cell migration, tissue organization, and development (Table 11).

Table 11. VEGF-VEGFR signaling and celluar chirality.

NR	PROPERTY	DESCRIPTION	REFERENCES
1	VEGF and Directional Migration:	VEGF gradients direct endothelial cell migration through VEGFR signaling, which guides cells to form structured, directional blood vessels. This directed movement is often chiral, showing bias in direction or rotation. Cellular chirality, a cell's intrinsic "handedness," influences how cells respond to VEGF cues, as cells with inherent chirality tend to migrate or align directionally in response to VEGF gradients. The interaction of VEGF signaling with chiral cues impacts collective cell migration, an essential step in forming vascular structures during development and wound healing	**[299]**
2	VEGFR and Actin Cytoskeleton in	VEGFR activation affects the actin cytoskeleton, which is integral to establishing and maintaining cell polarity and chiral behaviors. VEGF-driven actin reorganization enhances asymmetric cell migration through cellular protrusions	**[300]**

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	Chiral Migration:	(lamellipodia or filopodia) that are often biased in a particular direction. In some studies, VEGF has been shown to amplify cellular chirality in endothelial cells, coordinating their polarity and enhancing chiral migration during angiogenesis	
3	Cell Chirality and Tissue-Level Organization:	Cellular chirality, regulated by VEGF/VEGFR signaling, can result in coordinated chiral structures at the tissue level. During vascular development, VEGF/VEGFR-induced chiral migration facilitates the organization of endothelial cells into spiraling or coiling structures essential for vascular patterning and functionality. This is especially pertinent in the development of cardiac and brain structures, where vascular and cellular chirality must be meticulously synchronized to facilitate organ asymmetry and effective blood circulation.Cellular chirality, regulated by VEGF/VEGFR signaling, can result in coordinated chiral structures at the tissue level. During vascular development, VEGF/VEGFR-induced chiral migration facilitates the organization of endothelial cells into spiraling or coiling structures essential for vascular patterning and functionality. This is especially pertinent in the development of cardiac and brain structures, where vascular and cellular chirality must be meticulously synchronized to facilitate organ asymmetry and effective blood circulation.	**[301]**
4	VEGF in Developmental Left-Right Asymmetry:	Studies in developmental biology indicate that VEGF signaling might interact with pathways that establish left-right (LR) asymmetry in embryonic development, where cellular chirality plays a key role. During early organ development, VEGF/VEGFR expression in cells such as mesodermal and endothelial cells helps drive the asymmetric arrangement of organs, including the heart and lungs, where coordinated cellular chirality and directed vascularization are essential.	**[302]**
5	Cytoskeletal Dynamics and VEGFR Interactions	VEGFR activation often influences cytoskeletal reorganization, promoting the formation of actin structures like lamellipodia and filopodia. These structures are essential for directed cell migration and can display chiral movement patterns. Cytoskeletal remodeling regulated by VEGF/VEGFR and downstream effectors like Rho GTPases contributes to the chirality in cell migration, helping cells orient in specific directions during angiogenesis.	**[303]**
6	VEGF Gradient Formation and Directional Chirality	VEGF gradients play a significant role in directional cell migration, which is essential for chiral organization in vascular structures. Cells exposed to VEGF gradients respond by activating VEGFRs on the cell surface, which helps orient cell polarity and contributes to chiral migration patterns during angiogenic sprouting.	**[304]**
7	VEGF-Mediated Chiral Cell Polarity and Planar Cell Polarity (PCP) Pathway	VEGF signaling has been shown to interact with the planar cell polarity (PCP) pathway, which regulates cell orientation and alignment in tissues. PCP components help establish chiral orientations in endothelial cells, leading to coordinated chiral patterns in blood vessel formation. This pathway ensures cells orient correctly in response to VEGF signaling, promoting polarized cell structures.	**[305]**

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8	Interaction with Integrins for Coordinated Chiral Migration	Integrins work in coordination with VEGFRs to reinforce cell adhesion to the extracellular matrix (ECM), which enhances cellular alignment and polarity. Integrin-VEGFR crosstalk strengthens cell-ECM interactions necessary for the formation of chiral migration patterns during angiogenesis, particularly where cells need stable adhesion to move directionally.	**[306]**
9	Role of Mechanical Forces and Shear Stress on VEGF Signaling and Chirality	Shear stress due to blood flow regulates VEGF/VEGFR expression and aligns endothelial cells in a chiral, directional manner. This mechanotransduction reinforces chiral polarity and migration, especially in vascular tissues where cells respond to fluid dynamics.	**[307]**

These factors—cytoskeletal dynamics, VEGF gradients, planar cell polarity, integrin interactions, and mechanical forces—work alongside VEGF/VEGFR signaling to enhance cellular chirality. They promote directional migration, cell polarity, and organization within tissues, which are critical for developmental processes and tissue remodeling. By understanding how these pathways interact, researchers can better understand and potentially manipulate cellular behavior in contexts like angiogenesis, wound healing, and tissue engineering.

Furthermore, angiogenesis shaping methodologies using cellular /tissular indices are presented.

2.11. Angiogenesis shaping using cellular /tissular indices.

Shaping angiogenesis through cellular and tissue indices involves understanding and manipulating various factors that influence blood vessel formation. In the following table, the following methodologies are summarized (Table 12).

Table 12. Angiogenesis Shaping Using Cellular /Tissular Indices.

Nr	CELLULAR INDICES	TISSUE INDICES
1	**Endothelial Cell Density**: Measures the number of endothelial cells in a given area. Higher density typically indicates active angiogenesis.	Oxygen Tension (pO2): Hypoxia is a potent stimulus for angiogenesis. Measuring tissue oxygen levels can guide interventions in ischemic tissues.
2	**Proliferation Markers**: Proteins like Ki-67 or PCNA can indicate cell proliferation rates. Increased expression correlates with angiogenic activity.	**pH Levels**: The acidity of the microenvironment can influence cellular behavior and angiogenic responses. Analyzing tissue pH can help in understanding disease states.
3	**Migration Assays**: Evaluating the ability of endothelial cells to migrate towards a gradient of angiogenic factors helps understand their responsiveness to stimuli.	**Extracellular Matrix (ECM) Composition**: The types and organization of ECM components (like collagen fibronectin) affect angiogenesis. Changes in ECM composition can be indicative of disease progression.
4	**Tube Formation Assays**: Assessing the ability of endothelial cells to form capillary-like structures in vitro is a direct measure of angiogenic potential.	**Vascular Density**: Quantifying the number of blood vessels per unit area in a tissue sample provides a direct measure of angiogenic activity.
5	**Gene Expression Profiles**: Analyzing the expression of genes involved in angiogenesis (like VEGF, FGF) provides insight into cellular responses to various conditions.	**Inflammatory Markers**: Assessing levels of pro-inflammatory cytokines (like IL-1, TNF-α) can give insight into the tissue's angiogenic response, as inflammation often drives angiogenesis.

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6	**Endothelial Cell Senescence Markers**: Markers like p16INK4a and telomerase activity can indicate the aging status of endothelial cells, influencing their angiogenic potential.	**Mechanical Properties**: The stiffness or elasticity of tissue can influence angiogenesis. Stiffer matrices may promote vascularization, while softer ones may inhibit it.
7	**Endothelial Cell Senescence Markers**: Markers like p16INK4a and telomerase activity can indicate the aging status of endothelial cells, influencing their angiogenic potential.	**Vascular Endothelial Growth Factor (VEGF) Levels**: Measuring VEGF concentrations in tissues can indicate angiogenic activity, as it is a key regulator of blood vessel formation.
8	**Angiogenic Factor Expression**: Levels of factors such as VEGF, FGF, and angiopoietins are crucial for assessing the pro-angiogenic state of cells.	**Vascular Density (VD):** Quantifying the number of blood vessels per unit area in a tissue sample, often assessed through histological techniques.
9	**Adhesion Molecule Expression**: The presence of molecules like ICAM-1, VCAM-1, and E-selectin on endothelial cells can indicate their readiness to interact with leukocytes and other cells, influencing angiogenesis.	**Microvessel Density (MVD**): A specific measure of the density of small blood vessels, typically used in tumor studies to assess angiogenesis.
10	**Signal Transduction Pathway Activity**: Assessing the activation of pathways like PI3K/Akt, MAPK/ERK, and Notch signaling can provide insights into the cellular mechanisms driving angiogenesis.	**Fibrosis Index**: Evaluating the extent of fibrosis in tissues can indicate chronic conditions that may affect angiogenesis, as fibrotic tissues may have altered blood supply.
11	**Nitric Oxide Production**: Measurement of nitric oxide levels can indicate endothelial cell function, as it plays a key role in vascular relaxation and angiogenesis.	**Lactate Levels**: Elevated lactate in tissues can indicate anaerobic metabolism due to insufficient blood supply, influencing angiogenic processes.
12	**Migration and Invasion Assays**: Evaluating the ability of endothelial cells to migrate and invade through a Matrigel matrix can indicate their angiogenic potential.	**Histological Scoring of Inflammation**: Assessing the presence of inflammatory cells and associated markers can help gauge the inflammatory microenvironment that often drives angiogenesis.
13	**Expression of Matrix Metalloproteinases (MMPs)**: The expression levels of MMPs (like MMP-2 and MMP-9) are critical for assessing the ability of cells to remodel the extracellular matrix during angiogenesis.	**Proteoglycan and Glycosaminoglycan Levels:** The presence and composition of these ECM components can influence endothelial cell behavior and angiogenesis.
14	**Cytokine Production**: evels of pro-inflammatory cytokines (like IL-6 and IL-8) produced by endothelial cells can influence angiogenesis and recruitment of other cells.	**Collagen Organization**: Analyzing the alignment and type of collagen in the extracellular matrix can provide insights into the structural support for angiogenesis.
15	**Apoptotic Markers:** Assessing markers of apoptosis (like caspases or Annexin V) can help determine the survival of endothelial cells in the context of angiogenesis.	**Tissue Growth Factor Levels:** Levels of transforming growth factor-beta (TGF-β) and other growth factors can influence angiogenic processes and tissue remodeling.
16	**Staining for Endothelial Cell Markers**: Immunostaining for specific markers such as CD31 or VE-cadherin can confirm endothelial cell identity and help quantify their presence.	**Mechanical Stiffness**: Measuring the stiffness of tissues can provide information on how mechanical properties influence endothelial cell behavior and angiogenesis.

Gerhardt, H., & Betsholtz, C. discuss endothelial tip cell migration and the role of cellular polarity in guiding angiogenic sprouting. It highlights cellular indices like directional migration and

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polarity vector analysis to examine tip cell dynamics in response to VEGF gradients. This article emphasizes the role of Directional Migration and Tip Cell Selection in Angiogenesis [308].

Vion, A.C. et al. analyze how primary cilia and VEGF interact in endothelial cells to drive chiral organization and vascular patterning. Using cellular orientation indices, it evaluates how endothelial cells align and migrate to shape vessel architecture. The role of Role of Cellular Alignment and Collective Migration in Vessel Formation is summarised in this article [309]

Chen, K.D. et al. explore how mechanical cues like shear stress influence VEGF-driven angiogenesis, focusing on endothelial cell alignment and chirality under flow conditions. The study uses alignment and orientation indices to quantify cellular response to shear forces, showing how these factors contribute to angiogenic vessel shaping, emphasizing the importance of Mechanical Forces and Shear Stress on Endothelial Chirality [310]

Lamalice, L., Le Boeuf, F., & Huot emphasize the role of actin dynamics and focal adhesion distribution in endothelial cell migration. Cellular indices, such as focal adhesion rotation and actin alignment, are used to study the orientation and migration of cells during blood vessel sprouting [311].

Carmeliet, P., in his review, covers how cellular morphology and polarity affect angiogenic sprout formation. It discusses the importance of cellular shape indices in assessing how cell elongation and polarity drive structured angiogenesis [312].

Hynes, R.O. shows how Integrins, in coordination with VEGFR, play a critical role in endothelial cell adhesion, alignment, and polarity during angiogenesis. This study details how integrin-VEGFR crosstalk is assessed using indices for cell polarity and alignment to understand vascular organization [313].

Angelini, T.E. et al. describe Collective Cell Behavior and Multi-cellular Swirling in Vascular Patterning. This paper investigates collective cell migration and multi-cellular swirling patterns, applying cellular indices to measure alignment and swirling chirality. These indices are critical for analyzing coordinated cell behavior that shapes organized vascular structures [314].

Avraamides, C.J., Garmy-Susini, B., & Varner, J.A., in their review, discusses how integrin-mediated adhesion to the extracellular matrix (ECM) modulates endothelial cell orientation and chiral migration. Using cellular indices like focal adhesion density and polarity indices, this study examines how integrin signaling reinforces cellular alignment and organizes vessel structures [315].

Phng, L.-K., & Gerhardt, H. explore how Notch and VEGF signaling coordinate endothelial tip cell selection and migration. Polarity and directional indices are used to quantify cell responses in a gradient, highlighting the importance of VEGF and Notch interactions in shaping chiral migration patterns in angiogenesis [316]

Etienne-Manneville, S., & Hall investigate how Rho GTPases, downstream of VEGFR activation, influence cell polarity, migration, and actin dynamics. Cellular indices related to cytoskeletal alignment and polarity are used to study the Rho-mediated directional movement of endothelial cells in forming vascular structures [317].

Levesque, M.J., & Nerem, R.M show that this classic study examines the effects of mechanical stress, particularly shear stress, on VEGF-stimulated endothelial cell alignment and collective orientation. Using cellular orientation and alignment indices, it quantifies endothelial cell behavior under fluidic conditions, which are essential for vascular patterning [318].

Nelson, C.M., & Bissell discuss the role of cell shape and curvature in angiogenesis and how the extracellular environment influences endothelial cell organization. Cell shape indices, along with polarity indices, are used to analyze how membrane curvature guides cell-cell interactions in sprouting angiogenesis [319].

Siekmann, A.F., & Lawson, N.Dexamines how Notch signaling through lateral inhibition affects cell alignment and tip-stalk cell dynamics in angiogenesis. By applying cellular indices like alignment and polarity indices, the researchers analyze the spatial organization of cells in a vessel sprout [320].

Bentley, K. et al. show how VE-cadherin dynamics impact endothelial cell rearrangements and chiral orientation during angiogenic sprouting. The study uses chirality and alignment indices to quantify how endothelial cells align and shift to form structured blood vessels [321].

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3. Conclusions

The vascular angiogenesis and subsequent morphogenesis process indicate a complicated molecular network. Additional research is required to delineate the relationships among the implicated compounds accurately. Concerning the VEGFR pathway, VEGFR2 appears to be a pivotal component. The principal components of the VEGFR pathway are the VEGF receptors VEGFR1, VEGFR2, and VEGFR3. Multiple variants of VEGF are under investigation for potential medicinal use. Investigating the NOTCH and VEGFR signaling pathways may enable the modulation, enhancement, or suppression of vascular morphogenesis.

Cellular chirality—the inherent "handedness" of cells in movement, structure, and organization—plays a pivotal role in angiogenesis, the process by which new blood vessels form from pre-existing ones. This chiral behavior is fundamental to the directional and patterned organization of endothelial cells during vascular development and remodeling. In angiogenesis, cellular chirality guides endothelial cells to adopt precise orientations and migration patterns, which are essential for creating functionally structured blood vessels that can supply tissues with the necessary nutrients and oxygen. Cellular chirality in this context is influenced by various factors, including VEGF/VEGFR signaling, mechanical forces, interactions with the extracellular matrix (ECM), and cytoskeletal dynamics.

Quantifying these complex behaviors requires a range of cellular and tissue indices that capture both individual cell characteristics and collective tissue-level patterns. Cellular indices, such as cell orientation, polarity, and migration direction, allow researchers to measure chiral behaviors at the single-cell level, providing insights into how individual endothelial cells align, polarize, and move directionally in response to angiogenic cues. Tissue-level indices, such as multi-cellular alignment, rotational chirality, and ECM alignment, complement these indices. These reveal emergent patterns as cells interact and self-organize into structured vascular networks.

Through this combined approach, cellular and tissue indices have proven essential in advancing our understanding of angiogenesis, from molecular signaling to multicellular dynamics. By systematically quantifying cell and tissue behavior, these indices enable a detailed exploration of the mechanisms by which cellular chirality shapes vascular development, guiding efforts in regenerative medicine, cancer research, and developmental biology. The integration of cellular and tissue indices thus provides a comprehensive framework for analyzing both individual and collective behaviors, ultimately illuminating the principles governing tissue organization and vascular health.

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