Growth Factors Regulation in Angiogenesis

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Cardiovascular disease (CVD) remains a major cause of morbidity and mortality all over the world. Angiogenesis is controlled by cell–cell and cell-extracellular matrix (ECM) interactions through crosstalk between vascular endothelial growth factor (VEGF) and Notch signaling mechanisms.

tissue engineering cardiac vascular networks growth factors

1. Regulation of Growth Factors in Angiogenesis

Novel vascular structures are regulated by the surrounding cells and modulated by secretion of platelet-derived growth factor (PDGF) and VEGF secreted by ECs and vascular smooth muscle cells [1]. Among the factors that impact EC activation status are proteins called cytokines. A tissue can dictate the cellular response to a given cytokine. Hence, cytokines are considered as specialized symbols in intercellular interaction. This interaction is influenced by three factors, including the concentration of other cytokines in the environment; chemical and biological interactions between ECM, cells, and cytokines; and the cytoskeleton [2].

The signal protein most commonly studied to influence angiogenesis are VEGF, acidic fibroblast growth factor (aFGF), and basic fibroblast growth factor (bFGF) [3]. VEGF and FGF-2 have been studied in vitro to positively regulate several endothelial cell functions, that includes cellular proliferation, migration, extracellular proteolytic activity, and tube formation [3]. In addition, although a myriad of factors have been demonstrated to be active in the experimental setting, they are not all relevant to the endogenous regulation of new blood vessel formation. On the list of molecules that are active during the phase of activation, VEGF meets most of the criteria of a vasculogenic or angiogenic factor.

Angiogenesis regulators may act either directly on ECs or indirectly by inducing the production of direct-acting regulators by inflammatory and other non-EC populations. Thus, in contrast to VEGF and FGF-2, which are direct endothelial cell mitogens, the cytokines transforming growth factor-β (TGF-β) and tumor necrosis factor-α (TNF-α) have been studied to inhibit EC growth in vitro and are therefore direct-acting negative regulators [4]. However, both TGF-β and TNF-α are angiogenic in vivo, and it has been demonstrated to induce angiogenesis indirectly by stimulating the production of direct-acting positive regulators from stromal and chemoattracted inflammatory cells; hence, TGF-β and TNF-α are considered to be indirect positive regulators [5]. TGF-β has also been proposed to be a potential mediator of the phase of resolution due to its capacity to inhibit endothelial cell proliferation and migration directly, reduce extracellular proteolysis, and promote matrix deposition in vitro. In vitro, TGF-β has also

been studied to promote the organization of single endothelial cells embedded in three-dimensional collagen gels into tubelike structures, further signifying its role in the phase of resolution [4].

Other cytokines that have been studied to regulate angiogenesis in vivo include HGF, EGF/TGF-α, PDGF-BB, interleukins (IL-1, IL-6, and IL-12), interferons, GM-CSF, PlGF, proliferin, and proliferin-related protein. Angiogenesis can also be regulated by a variety of noncytokine or nonchemokine factors, including enzymes (angiogenin and PD-ECGF/TP), inhibitors of matrix-degrading proteolytic enzymes (TIMPs) and of PAs (PAIs), extracellular matrix components/coagulation factors or fragments (thrombospondin, angiostatin, hyaluronan, and its oligosaccharides), soluble cytokine receptors, prostaglandins, adipocyte lipids, and copper ions. The roles of these bioactive molecules are summarized in **Table 1**.

Table 1. Bioactive molecules and their effects on vascularization of tissue engineered constructs.

Bioactive Molecules	Angiogenic Effects	Ref
VEGF	Facilitates EC migration and proliferation Regulates EC proliferation, migration, and survival; allows mobilization of BM-derived cells such as HSCs, and recruit SMCs for stabilization of vessel.	[6]
FGF	FGF-2 Enhances EC proliferation. bFGF facilitates the activation, proliferation, and migration of EPC; regulate vasculogenesis and the formation of immature primary vascular networks. FGF-2 Interacts with ECM molecules such as heparin, heparan sulfate proteoglycans (HSPGs); promotes EC response and neovascularization process. FGF-2 facilitates proliferation of ECs, SMCs; endothelial capillary formation	[6]
IGF-1	Facilitates formation of neovasculature from the endothelium of pre-existing vessels andInduces endothelial cell migration for vascularization Induces the activation of the PI3-kinase/Akt signaling pathway and expression of growth factors	[7]
PDGF	Promotes vessel maturation by recruitment of MSCs, pericytes, and SMCs. Facilitates remodeling by inducing collagenases secretion by fibroblasts. Increases VEGF production and promote angiogenesis Regulates the production of ECM molecules for basement membrane and blood vessel stabilization	[8]
TGF-β	Promotes EC migration, proliferation, and differentiation. Increases VEGF secretion by ECs; and PGF and bFGF expression by SMCs. Enhances angiogenesis. Facilitates vessel stabilization and maturation Stimulates ECM deposition	[9]
HGF	Induces VEGF secretion Promotes angiogenesis by ECs expression of VEGF.	[10]
TNF-α	Inhibits proliferation of endothelial cells; promotes angiogenesis	[9]

Bioactive Molecules	Angiogenic Effects	Ref
Angiopoietin	Facilitates TGF-β-induced differentiation of MSCs. Promotes vessel maturation Inhibits VEGF activity and facilitates EC-SMC interactions Enhnaces type IV collagen deposition Promotes EC proliferation Induces VEGF mediated angiogenic sprouting.	[8]
SDF-1	Facilitates vessel stabilization by recruitment of progenitors of SMCs Initiate vascular remodeling; upregulate metalloproteinases and downregulate angiostatin	[11]

Abbreviations: VEGF (Vascular Endothelial growth factor), FGF (Fibroblast growth factor), IGF-1 (Insulin-like growth factor), PDGF (Platelet-derived growth factor), TGF-ß (Transforming growth factor-beta), HGF (Hepatocyte-growth factor), SDF-1 (Stromal cell derived growth factor), MSCs (Mesenchymal stem cells), VSMCs (Vascular Smooth muscle cells), TNF-α (Tumor necrosis factor α), EC (Endothelial cells), PGF (Placental growth factor), IGF-1 (Insulin growth factor -1), SMCs (Smooth muscle cells), EC (Endothelial cells), HSPGs (Heparan sulfate proteoglycans), FGF-2 (Fibroblast growth factor -2), bFGF (basic Fibroblast growth factor), HSCs (Hematopoietic stem cells), BM (Bone marrow), PGF (Placental growth factor), ECM (Extracellular matrix).

) promote vascularization. A myriad of angiogenic growth factors such as VEGF, PDGF-BB, bFGF, hepatocyte growth factor (HGF), insulin-like growth factor (IGF), and TGF-β, have been widely studied to promote vascularization in pathological disease models [12]. All key angiogenic growth factors (VEGF, FGF-2, IGF, HGF, PDGF-BB, and TGF-β1) bind to specific sites in the ECM; their release kinetics are based on their binding affinity and the proteases action to cleave the ECM or the ECM-binding growth factor domain [12]. It has been demonstrated through in vitro and in vivo studies that insufficient angiogenic growth factor exposure can inhibit angiogenesis, and subsequently, overexpression of growth factor can inhibit the function of vascular smooth muscle cells and pericytes population and form immature and unstable vessels [13]. The dose and duration of growth factor release has been studied to play a critical role in therapeutic applications. Several strategies have been employed to control the release of growth factors from biodegradable scaffolds. For example, angiogenesis has been enhanced using heparin or heparan sulfate-mimetic molecules covalently crosslinked with the collagen type I scaffold via 1-ethyl-3-dimethyl aminopropyl carbodiimide (EDC) and N-hydroxysuccinimide (NHS) for release of heparin-binding growth factors [14]. Further, angiogenesis has been studied to be enhanced by the combination of VEGF and FGF with a heparin-immobilized scaffold compared with a single growth factor molecule. Biomaterials have also been functionalized using surface modification strategies or heparin-binding ECM domain addition. For example, sequestration of multiple growth factors (VEGF-A165, PDGF-BB, and BMP-2) can be achieved using a fibrin matrix covalently crosslinked with multifunctional recombinant fibronectin (FN) fragments, including both its 12th and 14th type III repeats (FN III12-14) and FN III9-10 for enhanced angiogenic effects [15]. Angiogenic growth factors can also be altered for enhanced binding affinity to biomaterials for enhanced affinity with growth factors. Sacchi et al. achieved covalently crosslinking of fibrin hydrogels with VEGF fused to a sequence derived from α_2-plasmin inhibitor (α_2-$PI_{1–8}$) for controlled VEGF release by enzymatic cleavage, that resulted in stable and functional angiogenesis [16].

Incorporation of short bioactive peptides onto 3D scaffolds has gained interest as an effective method to achieve vascularization. Several approaches have been employed to study the effects of the immobilized bioactive peptides on vascular network formation. Increased EC attachment, growth, and migration were achieved by incorporation of integrin to ECM derived short peptide adhesive sequences such as collagen (Arg-Gly-Asp (RGD)), laminin (e.g., Tyr-Ile-Gly-Ser-Arg (YIGSR) and Ser-Ile-Lys-Val-Ala-Val (SIKVAV)), and FN (e.g., RGD and Arg-Glu-Asp-Val (REDV)) that increased angiogenesis [17]. Hydrogel activation by functional RGD and REDV sequences in an elastin-like recombinamer-based hydrogel caused improved EC adhesion and in vivo angiogenic potential via general cell adhesion and specific endothelial cell adhesion.

Several strategies have been taken to deliver bioactive molecules from tissue engineered scaffolds that mimic those associated with angiogenesis. Although the delivery of single-factor soluble factors such as bFGF can induce EC proliferation [18], the delivery of combinatorial growth factors better mimic the complexity of the angiogenic process. Various studies have demonstrated controlled dose and duration of growth factor release from biodegradable materials. Heparin-binding growth factors, VEGF and FGF-2 delivered from heparin-immobilized scaffolds exhibited an increased degree of angiogenesis in comparison to individual growth factor response [19]. Multiple growth factors (VEGF-A165, PDGF-BB, and BMP-2) were sequestered using fibrin matrix covalently crosslinked with multifunctional recombinant fibronectin (FN) fragments (12th and 14th type III repeats (FN III12-14) and FN III9-10) and exhibited enhanced angiogenic effects in a mouse model of chronic wound healing [19]. In another example, Kuttappan et al. functionalized a nanocomposite fibrous scaffold with combinations of VEGF, FGF-2, and BMP2 for differential growth factor release [20] that resulted in increased tissue vascularization. Furthermore, since growth factor delivery based on scaffold degradation can lead to an initial burst release, it was shown that increasing the crosslinking density of gelatin could improve growth factor retention. Turner et al. achieved controlled release of VEGF or BMP2 based on the progressive proteolytic degradation of the scaffold using crosslinked gelatin microspheres. However, the non-specific degradation of the scaffold and non-uniform growth factor release necessitates the need for development of more advanced systems for optimal release [21].

Incorporation of different bioactive molecules in sequential layers of polymers; a technique called layer-by-layer (LBL), can be employed for sequential delivery of growth factors. The incorporation of bioactive molecules and action of matrix-degrading enzymes causes sequential delivery of growth factors. A Polycaprolactone (PCL) scaffold was developed with sequential layers of heparin and VEGF was developed. Long-term anti-thrombogenic effect of tissue engineered graft was achieved by initial burst release of VEGF, facilitated by ECM degrading enzyme metallopeptidase-2 (MMP-2) and controlled release of heparin [22].

An enzyme-sensitive linker to link pro-angiogenic molecules covalently to the scaffold has been studied to promote angiogenesis. Linking the linker sequence to a specific enzyme (e.g., MMPs, serine, or cysteine proteinases) regulates time-bound release as enzymes are produced by cells at specific times during differentiation or angiogenesis. Light, an external stimulus for smart drug-delivery platforms, has been studied in various biomedical applications including image-guided surgery, and the photopolymerization and -degradation of tissue engineering scaffolds, for the advantages of its noninvasive properties, high spatial resolution, temporal control, and simple to use [23]. Light-sensitive linkers have been used to covalently bind molecules, with UV or near infrared (NIR) light used to release “incorporated” biomolecules. Light-responsive delivery systems must possess high spatial and temporal regulation over drug release, employ nonionizing radiation, formed of biocompatible materials, and flexible to be tailored to the needed application [24].

Encapsulation is another technique for controlled release of bioactive molecules. It has the advantage of providing protection for growth factors, increasing their half-life. Studies have been performed to design a scaffold patterned with composite microspheres, with the spatiotemporal release of proteins [25]. Lai et al. employed the technique of encapsulation via nanofibers and gelatin nanoparticles to form a scaffold for sequential release of VEGF, PDGF, FGF, and EGF (epithelial growth factor). This resulted in enhanced endothelial cell proliferation and development of

vascular networks [26]. Various approaches have been employed for controlled local delivery of angiogenic growth factors, however, the limitation of their inherent inability to control the geometric architecture of vascular networks needs to be addressed for optimal 3D tissue construction. These techniques can be employed to control growth factor delivery recapitulating the temporal pattern observed in physiological angiogenesis, but with limited complexity. Despite the promise growth factor delivery or bioactive-peptide-guided vascular network formation, these approaches still lack the control network geometry, for generation of a spatially controllable 3D mature vascular network. Therefore, advancements in fabrication technologies below aim to fabricate spatially controllable 3D vascular networks using scaffolds.

References

1. Campinho, P.; Vilfan, A.; Vermot, J. Blood Flow Forces in Shaping the Vascular System: A Focus on Endothelial Cell Behavior. Front. Physiol. 2020, 11, 552.
2. Boyd, D.F.; Thomas, P.G. Towards integrating extracellular matrix and immunological pathways. Cytokine 2017, 98, 79–86.
3. Ucuzian, A.A.; Gassman, A.A.; East, A.T.; Greisler, H.P. Molecular Mediators of Angiogenesis. J. Burn Care Res. 2010, 31, 158–175.
4. Fallah, A.; Sadeghinia, A.; Kahroba, H.; Samadi, A.; Heidari, H.R.; Bradaran, B.; Zeinali, S.; Molavi, O. Therapeutic targeting of angiogenesis molecular pathways in angiogenesis-dependent diseases. Biomed. Pharmacother. 2018, 110, 775–785.
5. Pepper, M.S. Manipulating Angiogenesis. Arter. Thromb. Vasc. Biol. 1997, 17, 605–619.
6. Nazeer, M.A.; Karaoglu, I.C.; Ozer, O.; Albayrak, C.; Kizilel, S. Neovascularization of engineered tissues for clinical translation: Where we are, where we should be? APL Bioeng. 2021, 5, 021503.
7. Jacobo, S.M.P.; Kazlauskas, A. Insulin-like Growth Factor 1 (IGF-1) Stabilizes Nascent Blood Vessels. J. Biol. Chem. 2015, 290, 6349–6360.
8. Peters, E.B. Endothelial Progenitor Cells for the Vascularization of Engineered Tissues. Tissue Eng. Part B Rev. 2018, 24, 1–24.
9. Zhang, Y.; Liu, J.; Zou, T.; Qi, Y.; Yi, B.; Dissanayaka, W.L.; Zhang, C. DPSCs treated by TGF-β1 regulate angiogenic sprouting of three-dimensionally co-cultured HUVECs and DPSCs through VEGF-Ang-Tie2 signaling. Stem Cell Res. Ther. 2021, 12, 281.
10. Zhang, L.-L.; Xiong, Y.-Y.; Yang, Y.-J. The Vital Roles of Mesenchymal Stem Cells and the Derived Extracellular Vesicles in Promoting Angiogenesis After Acute Myocardial Infarction. Stem Cells Dev. 2021, 30, 561–577.

11. Dierick, F.; Solinc, J.; Bignard, J.; Soubrier, F.; Nadaud, S. Progenitor/Stem Cells in Vascular Remodeling during Pulmonary Arterial Hypertension. Cells 2021, 10, 1338.
12. Ren, X.; Zhao, M.; Lash, B.; Martino, M.M.; Julier, Z. Growth Factor Engineering Strategies for Regenerative Medicine Applications. Front. Bioeng. Biotechnol. 2020, 7, 469.
13. Chu, H.; Wang, Y. Therapeutic angiogenesis: Controlled delivery of angiogenic factors. Ther. Deliv. 2012, 3, 693–714.
14. Ikegami, Y.; Mizumachi, H.; Yoshida, K.; Ijima, H. Heparin-conjugated collagen as a potent growth factor-localizing and stabilizing scaffold for regenerative medicine. Regen. Ther. 2020, 5, 236–242.
15. Wang, Z.; Wang, Z.; Lu, W.W.; Zhen, W.; Yang, D.; Peng, S. Novel biomaterial strategies for controlled growth factor delivery for biomedical applications. NPG Asia Mater. 2017, 9, e435.
16. Martino, M.M.; Brkic, S.; Bovo, E.; Burger, M.; Schaefer, D.J.; Wolff, T.; Gürke, L.; Briquez, P.S.; Larsson, H.M.; Gianni-Barrera, R.; et al. Extracellular Matrix and Growth Factor Engineering for Controlled Angiogenesis in Regenerative Medicine. Front. Bioeng. Biotechnol. 2015, 3, 45.
17. Tallawi, M.; Rosellini, E.; Barbani, N.; Cascone, M.G.; Rai, R.; Saint-Pierre, G.; Boccaccini, A.R. Strategies for the chemical and biological functionalization of scaffolds for cardiac tissue engineering: A review. J. R. Soc. Interface 2015, 12, 20150254.
18. Hu, C.; Ayan, B.; Chiang, G.; Chan, A.H.P.; Rando, T.A.; Huang, N.F. Comparative Effects of Basic Fibroblast Growth Factor Delivery or Voluntary Exercise on Muscle Regeneration after Volumetric Muscle Loss. Bioengineering 2022, 9, 37.
19. Sedlář, A.; Trávníčková, M.; Matějka, R.; Pražák, Š.; Mészáros, Z.; Bojarová, P.; Bačáková, L.; Křen, V.; Slámová, K. Growth Factors VEGF-A165 and FGF-2 as Multifunctional Biomolecules Governing Cell Adhesion and Proliferation. Int. J. Mol. Sci. 2021, 22, 1843.
20. Kuttappan, S.; Mathew, D.; Jo, J.-I.; Tanaka, R.; Menon, D.; Ishimoto, T.; Nakano, T.; Nair, S.V.; Nair, M.B.; Tabata, Y. Dual release of growth factor from nanocomposite fibrous scaffold promotes vascularisation and bone regeneration in rat critical sized calvarial defect. Acta Biomater. 2018, 78, 36–47.
21. Turner, P.A.; Thiele, J.S.; Stegemann, J.P. Growth factor sequestration and enzyme-mediated release from genipin-crosslinked gelatin microspheres. J. Biomater. Sci. Polym. Ed. 2017, 28, 1826–1846.
22. Cuenca, J.P.; Kang, H.-J.; Al Fahad, A.; Park, M.-K.; Choi, M.-J.; Lee, H.-Y.; Lee, B.-T. Physico-mechanical and biological evaluation of heparin/VEGF-loaded electrospun polycaprolactone/decellularized rat aorta extracellular matrix for small-diameter vascular grafts. J. Biomater. Sci. Polym. Ed. 2022, 33, 1664–1684.

23. Damiri, F.; Kommineni, N.; Ebhodaghe, S.O.; Bulusu, R.; Jyothi, V.G.S.S.; Sayed, A.A.; Awaji, A.A.; Germoush, M.O.; Al-Malky, H.S.; Nasrullah, M.Z.; et al. Microneedle-Based Natural Polysaccharide for Drug Delivery Systems (DDS): Progress and Challenges. Pharmaceuticals 2022, 15, 190.
24. Sagar, V.; Nair, M. Near-infrared biophotonics-based nanodrug release systems and their potential application for neuro-disorders. Expert Opin. Drug Deliv. 2017, 15, 137–152.
25. Pandolfi, L.; Minardi, S.; Taraballi, F.; Liu, X.; Ferrari, M.; Tasciotti, E. Composite microsphere-functionalized scaffold for the controlled release of small molecules in tissue engineering. J. Tissue Eng. 2016, 7, 2041731415624668.
26. Lai, H.-J.; Kuan, C.-H.; Wu, H.-C.; Tsai, J.-C.; Chen, T.-M.; Hsieh, D.-J.; Wang, T.-W. Tailored design of electrospun composite nanofibers with staged release of multiple angiogenic growth factors for chronic wound healing. Acta Biomater. 2014, 10, 4156–4166.