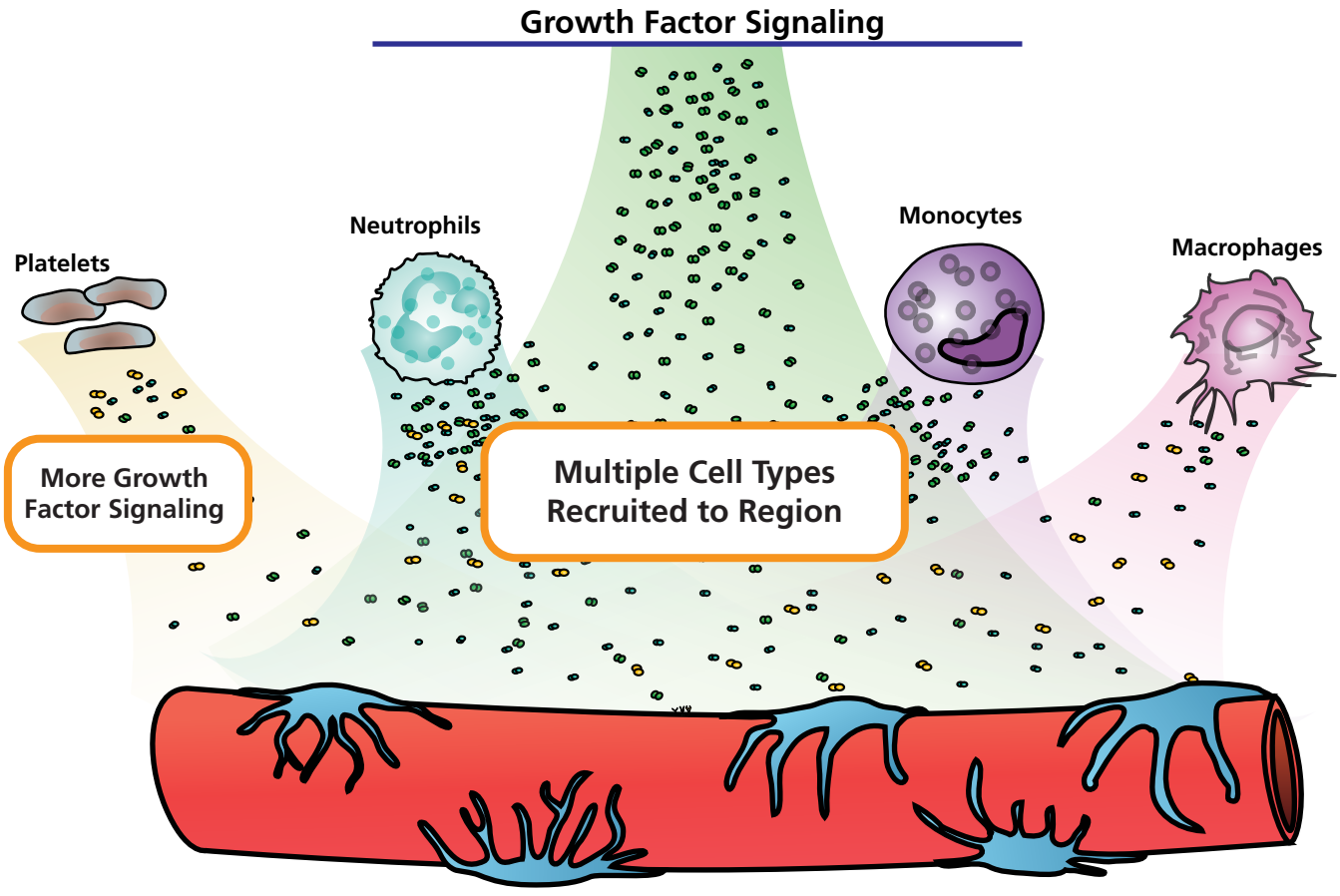
MODULE 4.2
Stimulating Vessel Growth

Angiogenesis - the growth of new blood vessels - is an important natural process occurring in the body, both in health and in disease. Angiogenesis occurs in the healthy body during wound healing and for restoring blood flow to tissues following ischemic injury or insult. In women, angiogenesis also occurs during the menstrual cycle and during pregnancy. In the healthy body, a series of biologic "on" and "off" switches help modulate angiogenesis. The main "on" switches are known as *angiogenesis-stimulating growth factors* whereas the main "off" switches are known as *angiogenesis inhibitors*.

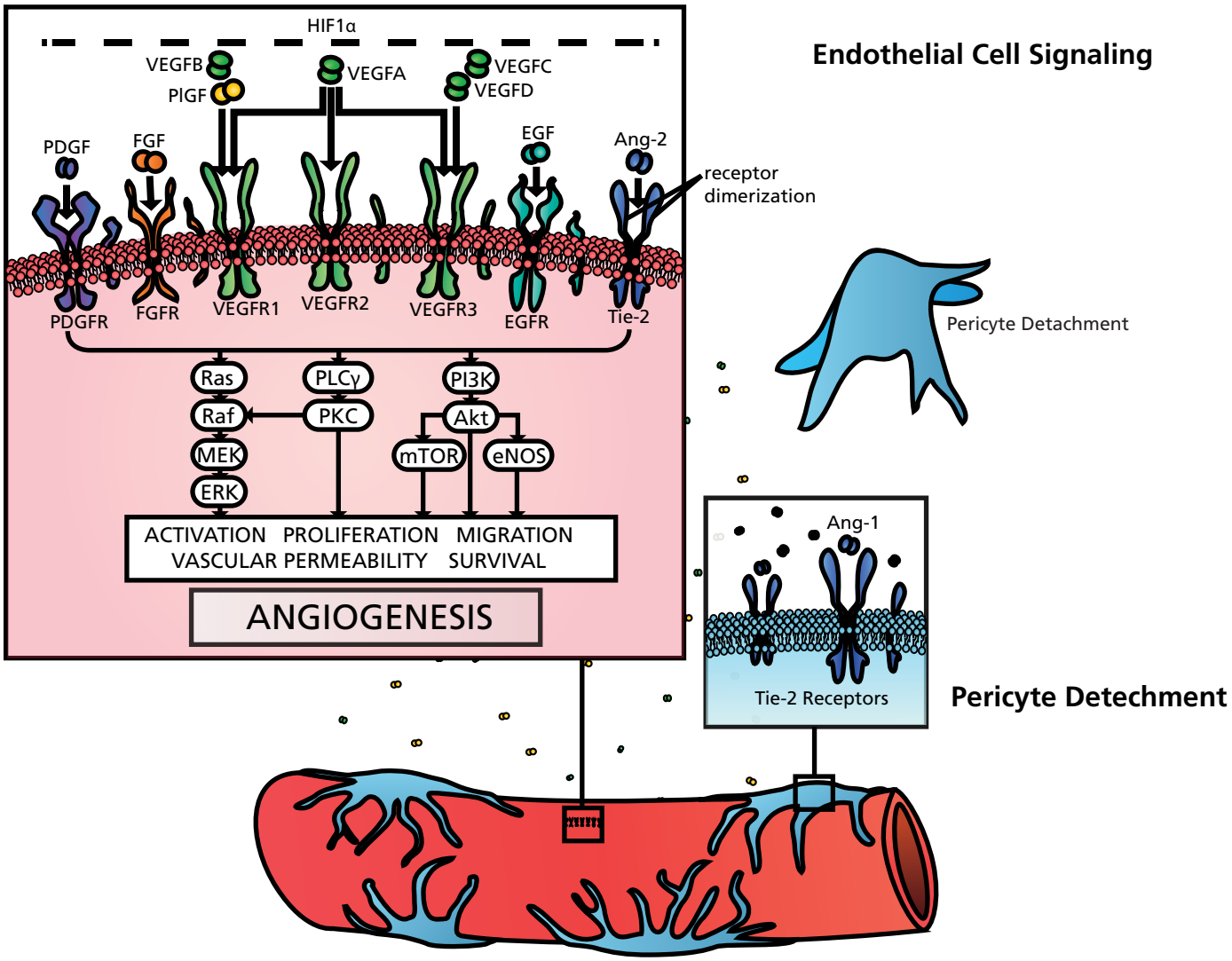
When angiogenic growth factors are produced in excess of angiogenesis inhibitors, the balance is tipped in favor of blood vessel growth. When inhibitors are present in excess of stimulators, angiogenesis is stopped. When a quiescent vessel senses an angiogenic signal, such as vascular endothelial growth factor (VEGF), several processes are initiated concomitantly. The binding of VEGF to VEGF receptor-2 (VEGFR-2) activates endothelial dells (ECs) and induces their proliferation and migration. At the same time, matrix metalloproteinases (MMPs) are activated and their inhibitors (tissue metalloproteinases 1 and 2; TIMP-1 and TIMP-2) are suppressed. This leads to proteolytic degradation of the basement membrane (BM), and consequently, release of angiogenic molecules, such as VEGF and fibroblast group factor (FGF), that are stored in the extracellular matrix (ECM) - adding to the local angiogenic milieu. Angiopoietin-2 (ANG-2), a proangiogenic growth factor stored in ECs for rapid release, causes the detachment of pericytes from the vessel wall, leading to further destabilization of the vessel. At the same time, ECs loosen their cell-cell junctions, and the nascent vessel dilates.[1-3]

Sprouting of the vessel is initiated following the selection of one of the ECs as a tip cell, which migrates and leads the edge of the newly forming capillary. The selection is based on the overall combined effects of VEGFs, their receptors, neuropilins (NRPs), and the Notch ligands, DLL_4 and $JAGGED_1$. The neighbors of the tip cell become stalk cells, which divide to elongate the sprout (stimulated by Notch, Notch-regulated ankyrin repeat protein [NRARP], Wnts, placental growth factor [PlGF], and

1. Initiation: Hypoxia, Metabolic Switch



2. Receptor Activation & Cell Signaling



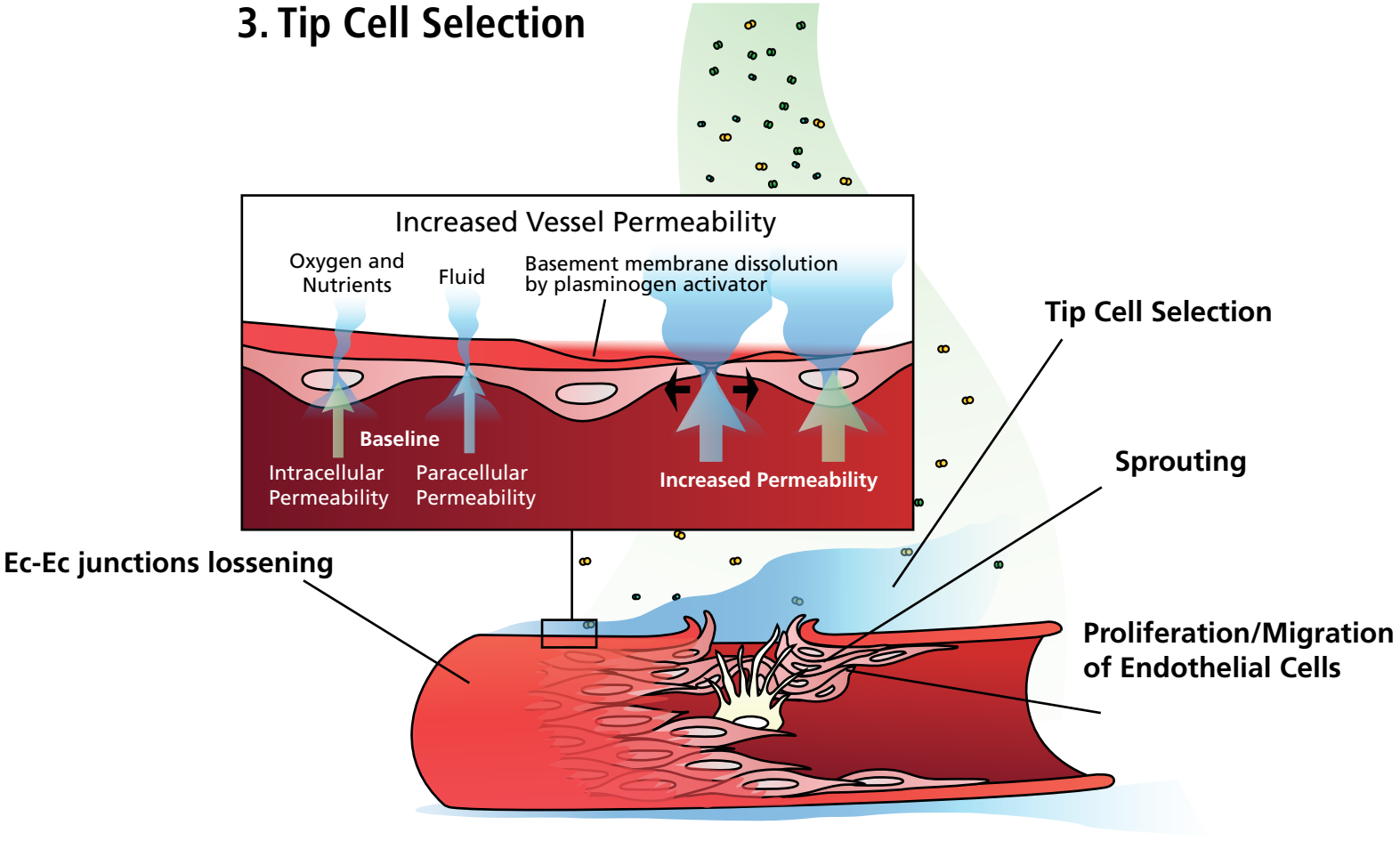
FGFs) and establish the lumen.[3,4] As the new sprout grows in length, tip cells constantly release MMPs that dissolve the BM and the ECM and use filopodia to push the edge of the cell towards the angiogenic stimuli, while the stalk cells keep proliferating and elongating. When the edges of two sprouts connect and their leading tip cells fuse, a new continuous tube is formed (vessel anastomosis). At this stage, the vessel lumen begins to form and blood starts to flow.[5,6] This stage is mediated by macrophages and is accompanied by the deposition of ECM molecules into the BM, the recruitment of supporting pericytes, reduced EC proliferation, and increased formation of cell-cell junctions.

Initially, sprouts occur in high numbers to form a massive number of tree-like branching formations (known as arborization).[7-9] This tortuous, dense growth pattern needs to be remodeled and pruned back according to the tissue's needs.

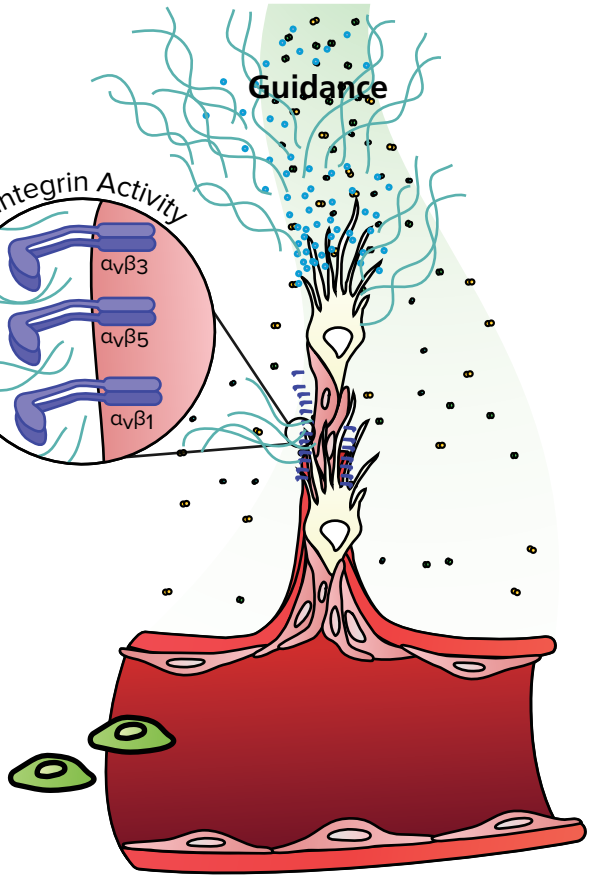
Vessel remodeling involves pruning (regression) of unnecessary new sprouts and of vessels that are unable to become perfused.[3,4] To become fully functional, the remaining vessels must become mature and stable. This involves the transition of ECs from proliferation to a quiescent state. ECsresume their quiescent phalanx state, and signals such as platelet-derived growth factor B (PDGF-B), angiopoietin 1 (ANG-1), transforming growth factor-β (TGF-β), ephrin-B_2, and Notch lead to perictye attachment to and cover of ECs. Pericytes, in turn, exert a stabilizing effect on newly formed vessels and arrest their growth. Protease inhibitors (such as TIMPs and plasminogen activator inhibitor-1 [PAI-1]) enhance the deposition of a BM, and ECs' cell-cell junctions are reestablished to ensure optimal flow distribution.[3,8,10] After the completion of a successful process of functional blood vessel formation, the angiogenesis process must be completely shut down. The decreased oxygen demand and decline in tissue hypoxia result in a diminished production and activity of soluble proangiogenic mediators.

Endogenous angiogenesis inhibitors such as thrombospondin, endostatin, angiostatin, tumstatin, canstatin, vasostatin, and pigment epithelium-derived factor (PEDF) are produced around the vessels and help restore the normal state of quiescence [9].

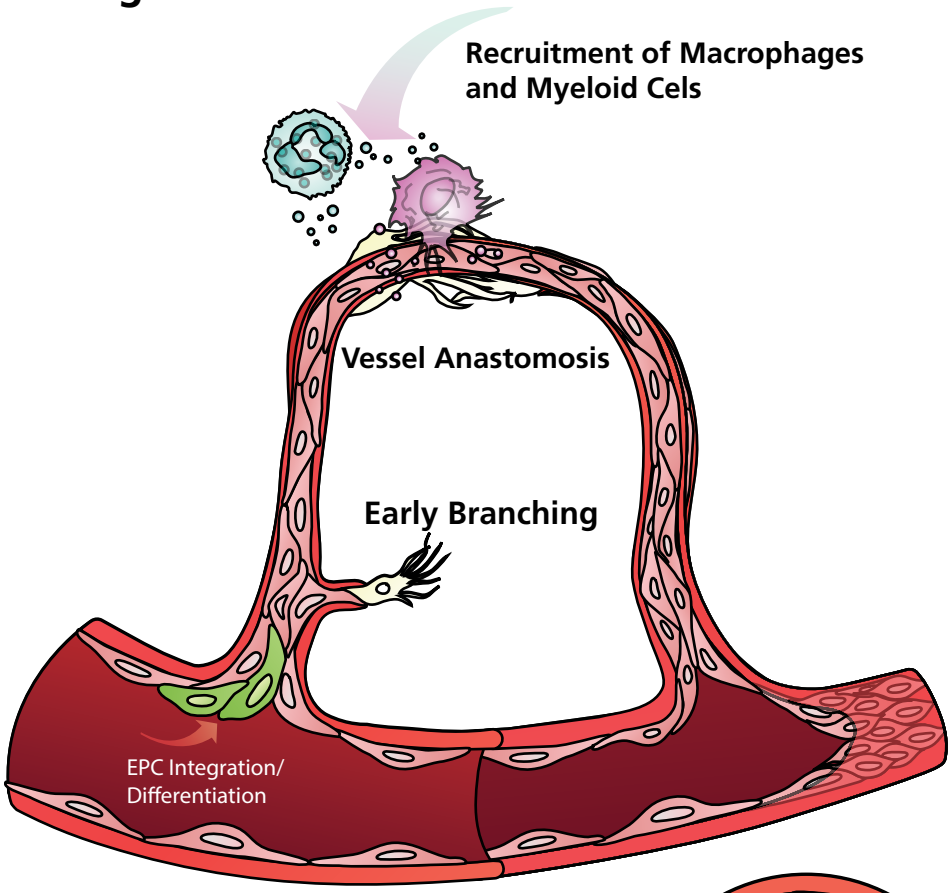
3. Tip Cell Selection



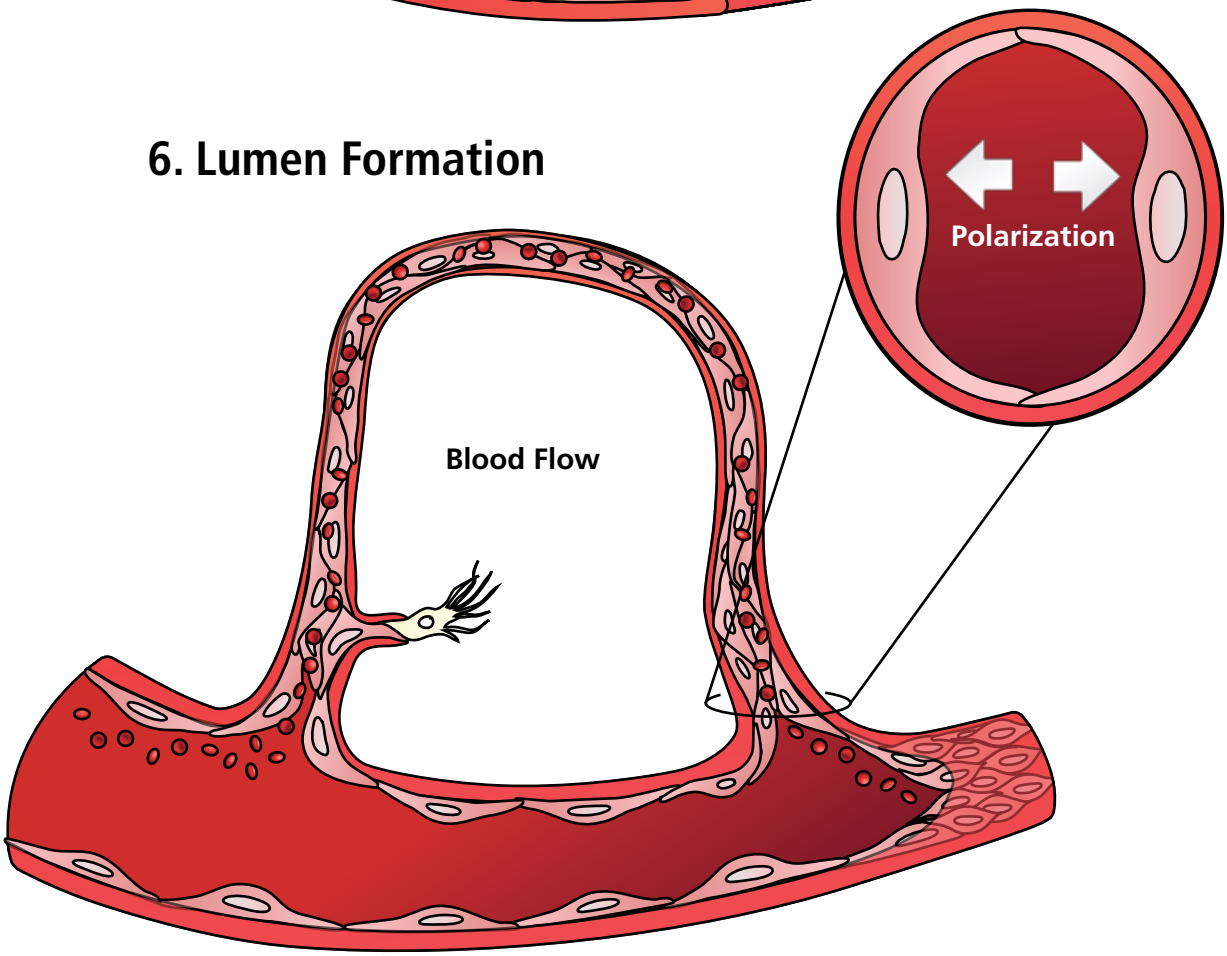
4. Sprouting, Invasion



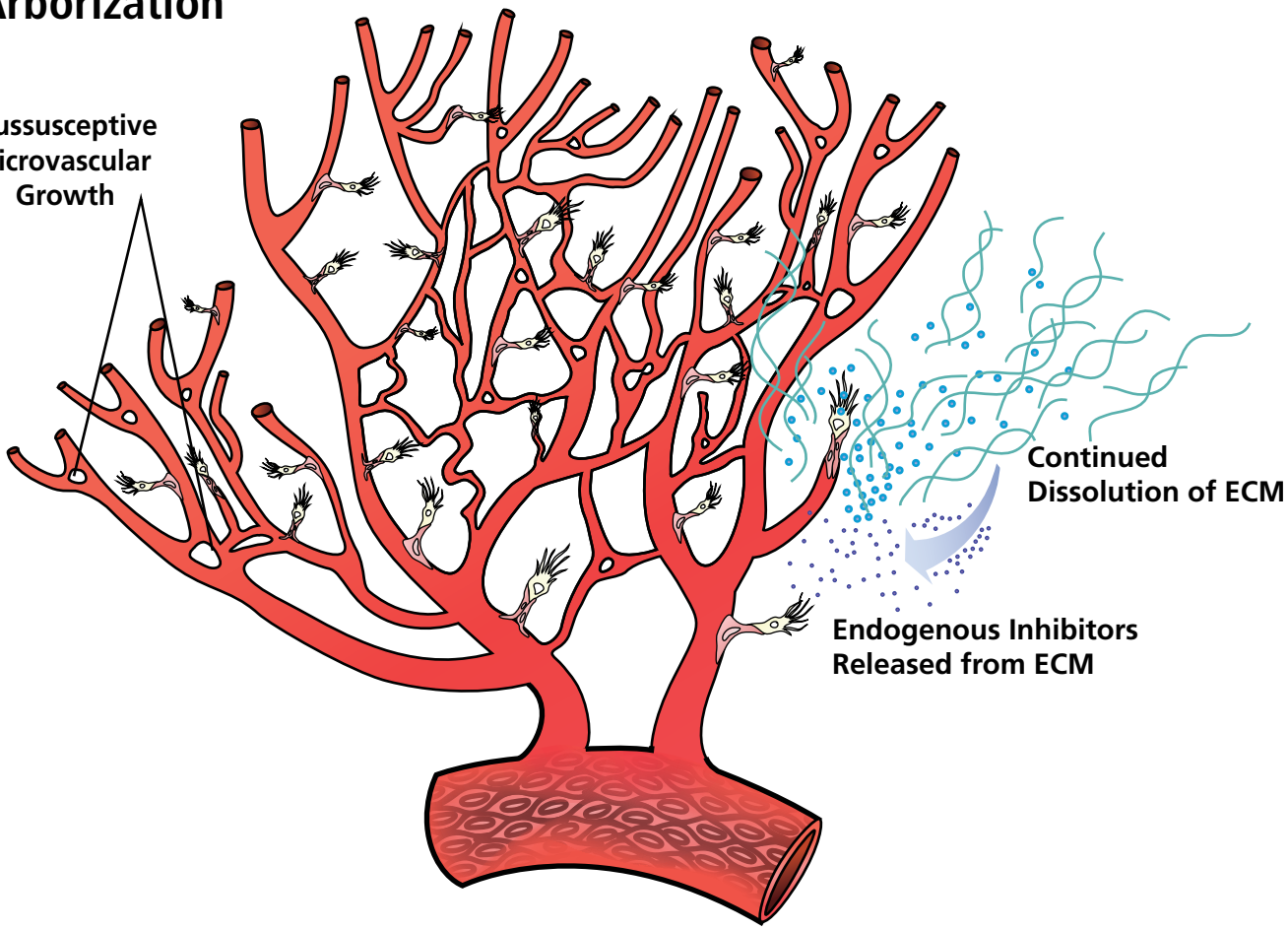
5. Sprouting Connections



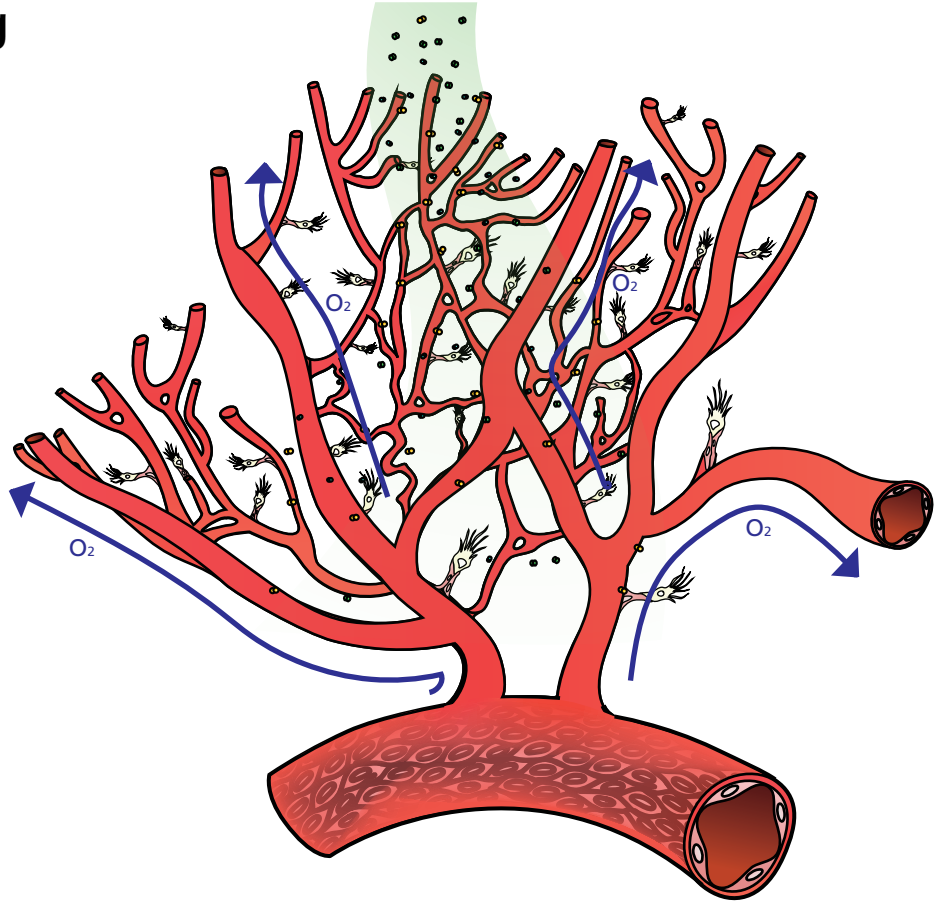
6. Lumen Formation



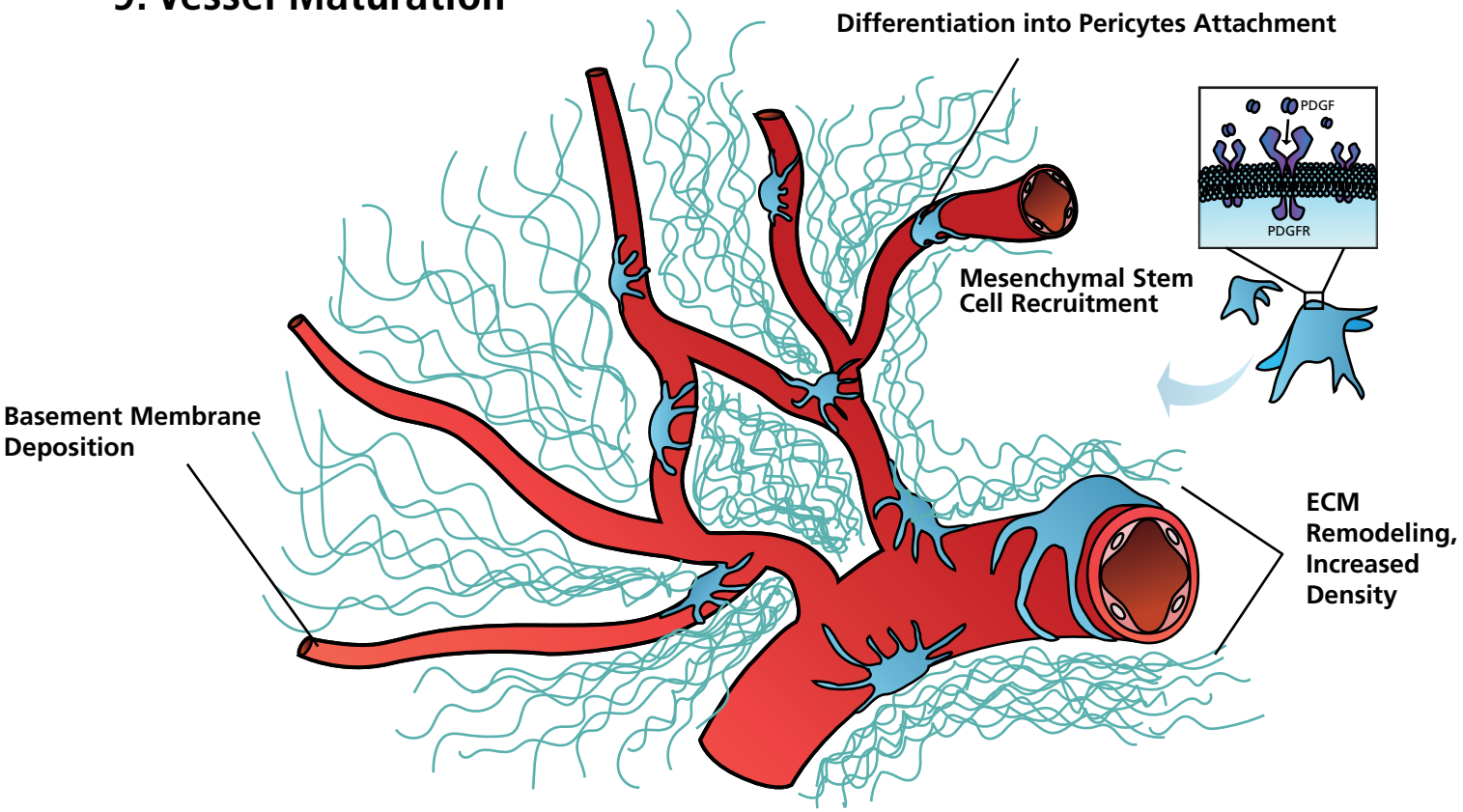
7. Vessel Arborization



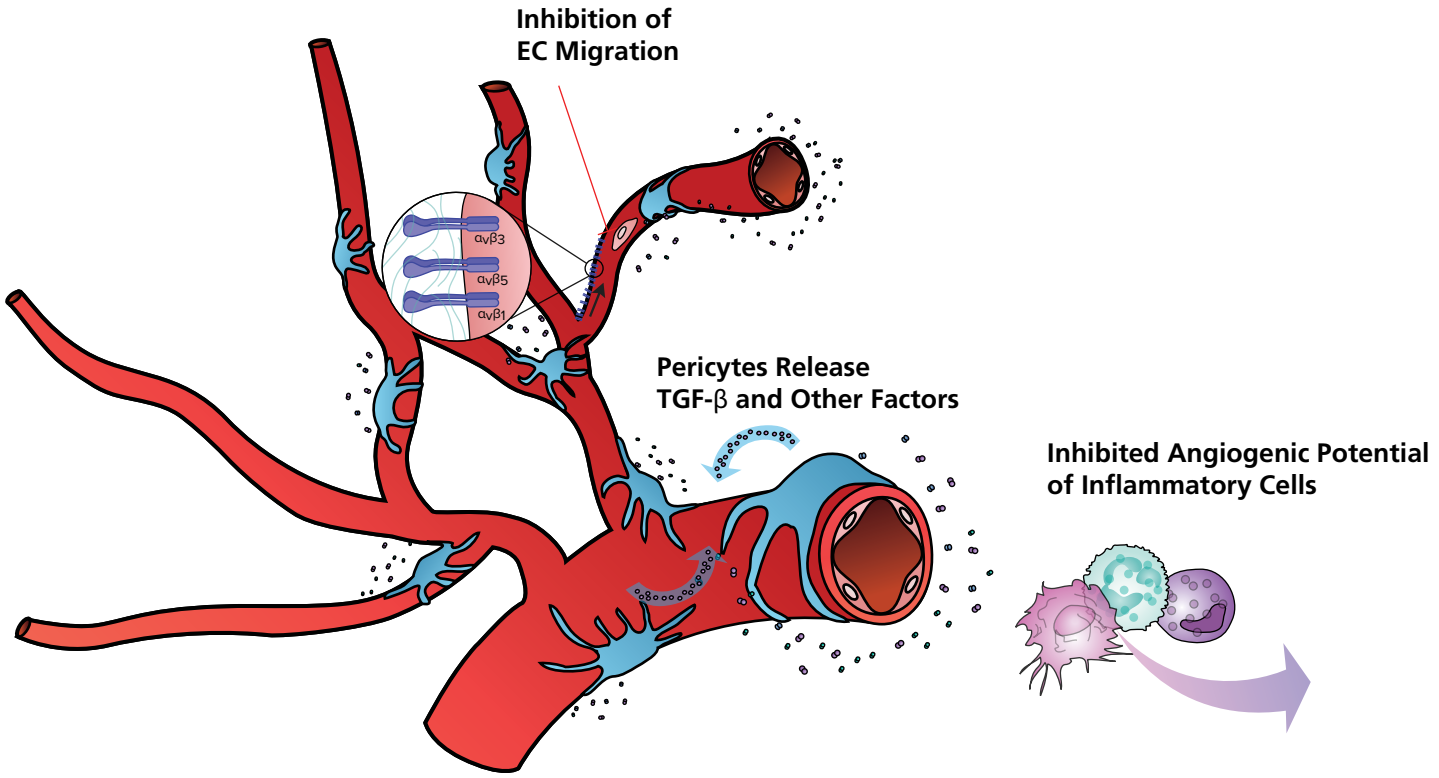
8. Vessel Pruning



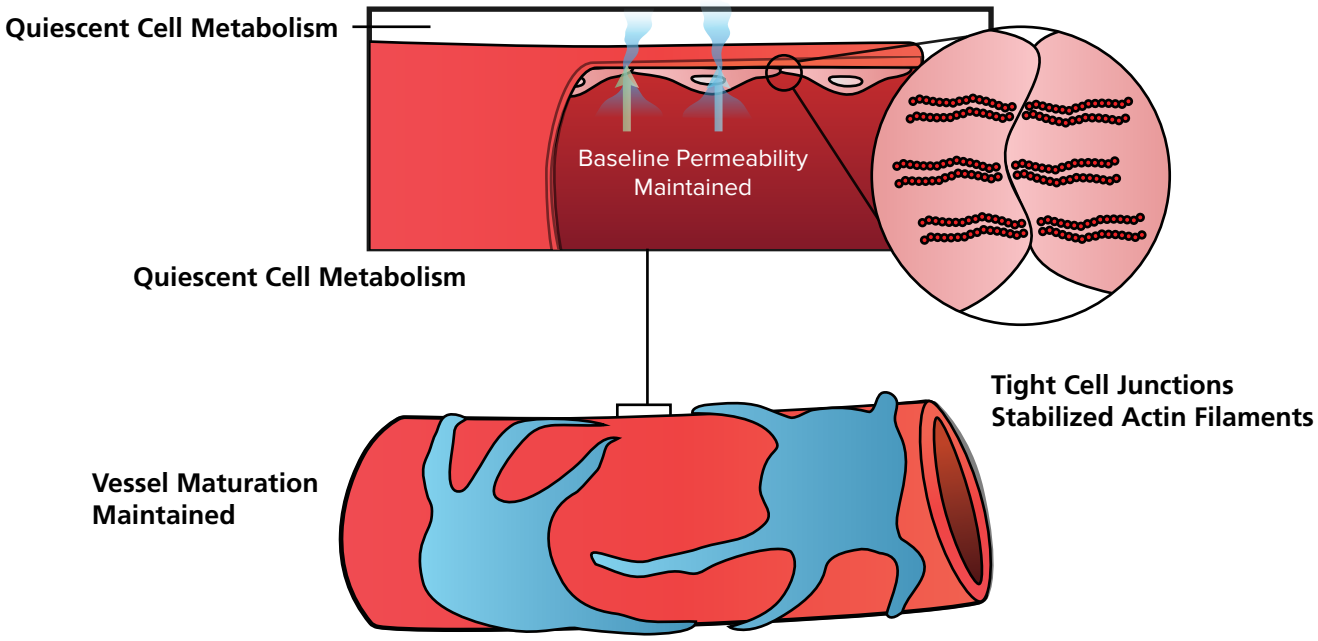
9. Vessel Maturation



10. Return to Quiescence



11. Maintain Quiescence



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