White Paper

PromoCell®

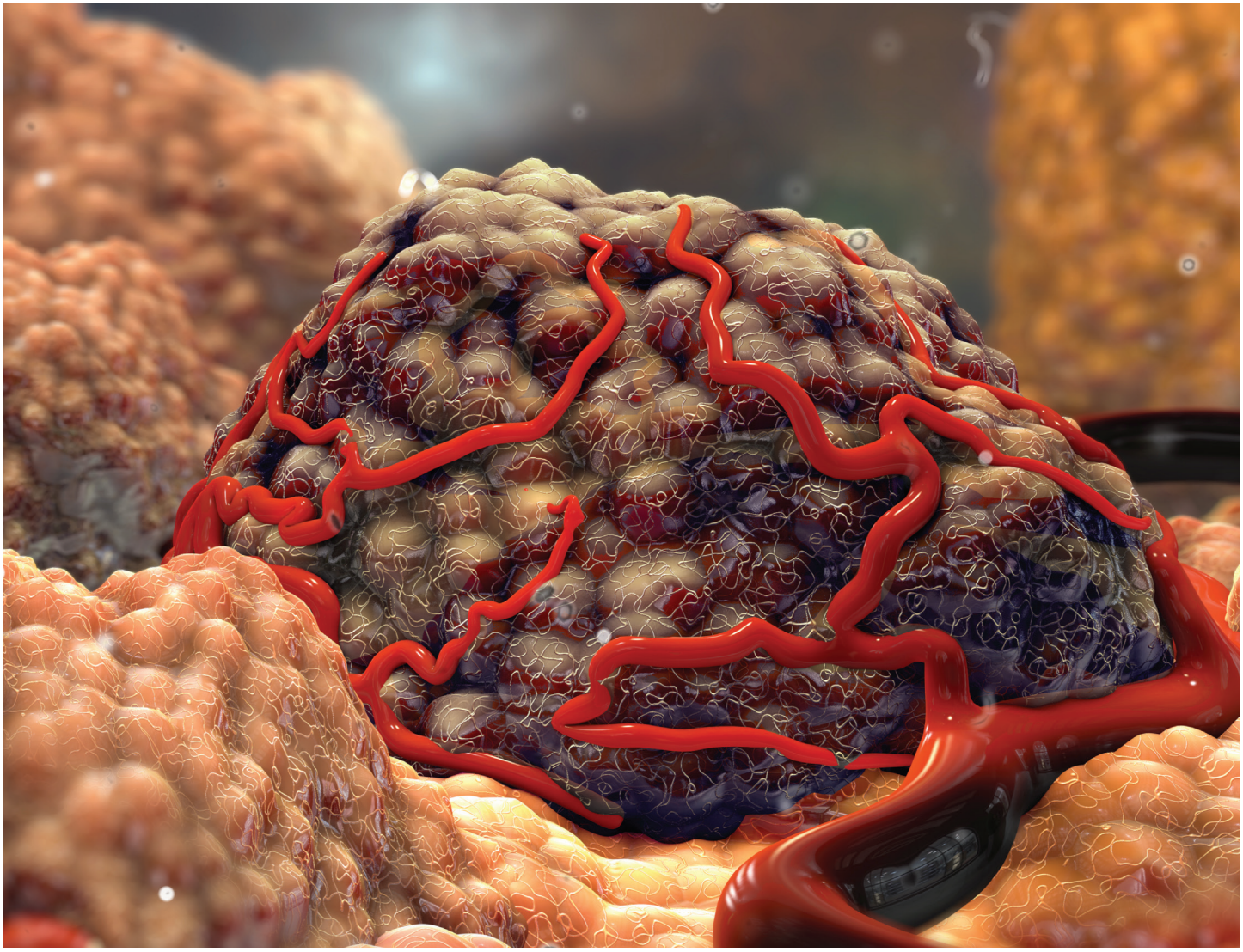
The Role of Angiogenesis in Cellular Processes and Disorders

What is Angiogenesis?

Angiogenesis is the physiological development of new blood vessels from existing ones – an essential process for embryonic and fetal development, organ and skeletal growth, wound healing, and the menstrual cycle.

Physiological angiogenesis is a highly organized sequence of cellular events, triggered by tissue hypoxia or insufficient oxygen tension, and is highly regulated and balanced by pro- and anti-angiogenic factors. Pathological angiogenesis however is less well controlled, in this state vessels rarely mature, remodel or regress in response to disease. Understanding angiogenesis more thoroughly will give researchers insights into new therapies and tissue engineering. While scientists have already made significant process in understanding angiogenesis, it is a complex process that is highly regulated, meaning there are many factors still to understand. Various assays are available to study the process, which we will cover in more detail below.

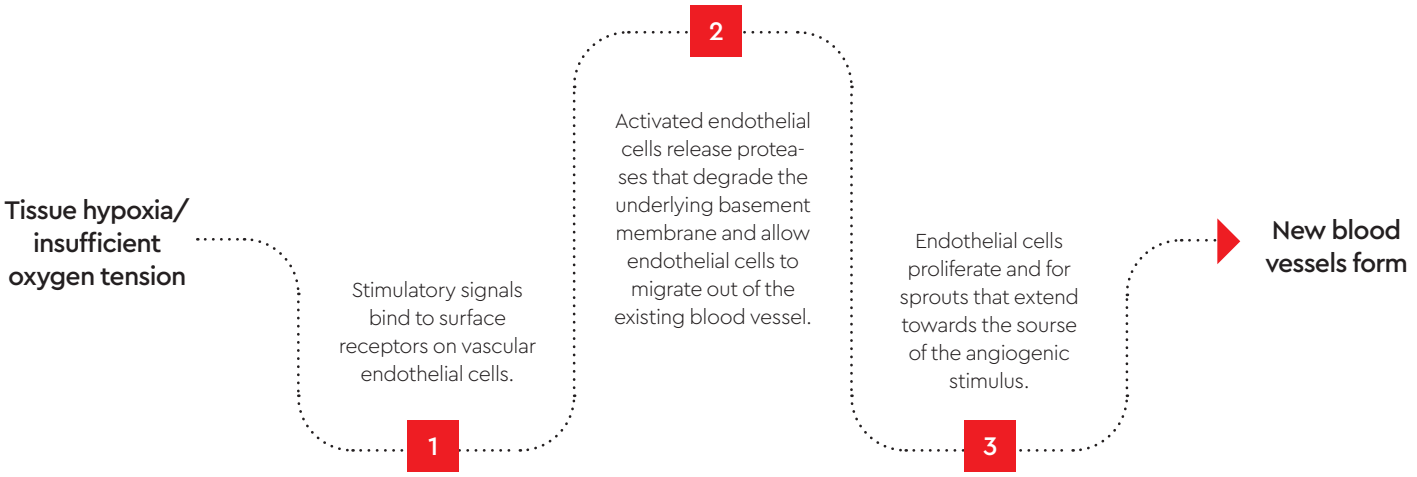
Read on to discover more about how angiogenesis works, what causes angiogenesis to occur and the assays you can use to study angiogenesis.



The Process of Angiogenesis

While there are three processes that occur in the body to form new blood vessels (vasculogenesis, arteriogenesis, and angiogenesis), angiogenesis is the only process that occurs in adults. While angiogenesis typically occurs during processes we think of as growth (such as fetal development) it is also an integral part of the menstrual cycle. Once angiogenesis has occurred, newly formed blood vessels lined with endothelial cells enable immune surveillance and removal of waste products.

The process of physiological angiogenesis occurs in a highly organized and regulated sequence of events. These include vascular initiation, sprouting, formation, maturation, remodeling and regression. Each stage is regulated to meet the requirements of the tissue angiogenesis is occurring in.



Types of Angiogenesis?

Sprouting angiogenesis was discovered around 200 years ago and is characterized by sprouts made up of endothelial cells that typically grow towards an angiogenesis stimulus like vascular endothelial growth factor A (VEGF-A). This kind of angiogenesis can enrich previously vessel-free tissue with blood vessels.

Intussusceptive angiogenesis was only discovered around twenty years ago and involves the splitting of interstitial tissues to form transvascular tissue pillars.

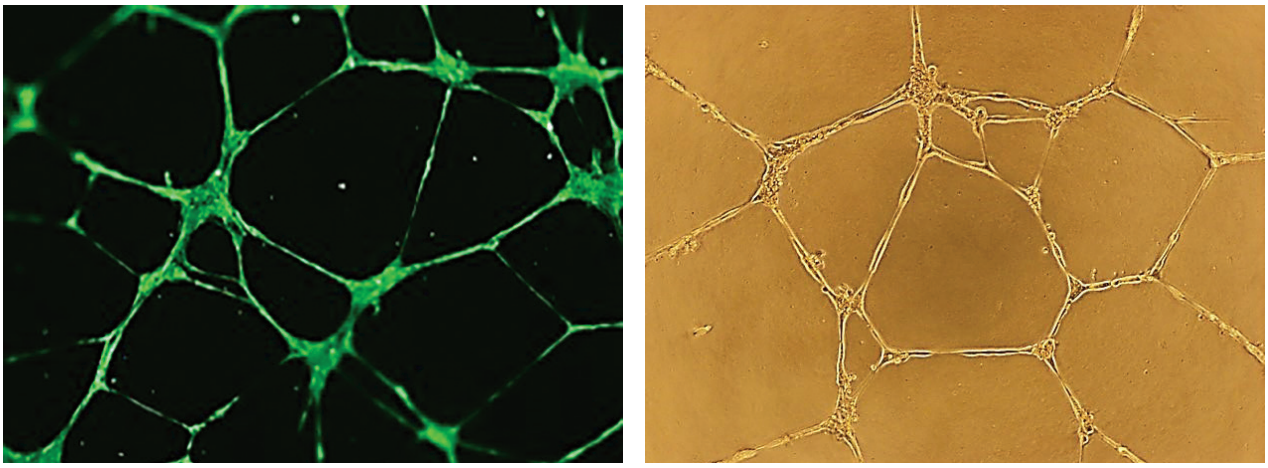


Figure 1: *Image analysis of endothelial cell tube formation.* ***(Left)*** *Light microscopy image of PromoCell HUVECs cultured on Basement Membrane Extract for 17 hours.* ***(Right)*** *Calcein staining of PromoCell HUVECs cultured on Basement Membrane Extract for 17 hours using PromoCells's Angiogenesis Assay Kit.*

Anti-angiogenic Factors

Other factors inhibit angiogenesis. These include:

- Angiostatin
- Endostatin
- Interferon
- Platelet factor 4
- Thrombospondin-1 protein
- Prolactin
- Interleukin-12

Angiogenesis in Disease

While angiogenesis is a necessary physiological process, it is also involved in various diseases. In fact, it is the hallmark process of many diseases, in which angiogenesis should not be occurring under physiological conditions. While physiological angiogenesis is highly controlled, pathological angiogenesis is less so – the vessels often do not mature, remodel or regress. The 'angiogenic switch' refers to the balance between angiogenic and anti-angiogenic factors, when unbalanced this can lead to disease. Over-proliferation of blood vessels caused by prolific angiogenesis is involved in several disorders, including:

- Hypertension and atherosclerosis
- Diabetes and obesity
- Asthma and arthritis
- Psoriasis, dermatitis and lupus
- Cancer

Of significance, cancers require the formation of new blood vessels to grow and metastasize, therefore cancerous cells secrete substances that stimulate angiogenesis. When travelling to new sites, metastatic cells bring new blood vessels with them to enable growth in their new locations. A blood supply is essential to the survival of cancerous tissue, and therefore of great interest to researchers looking for cancer therapies.

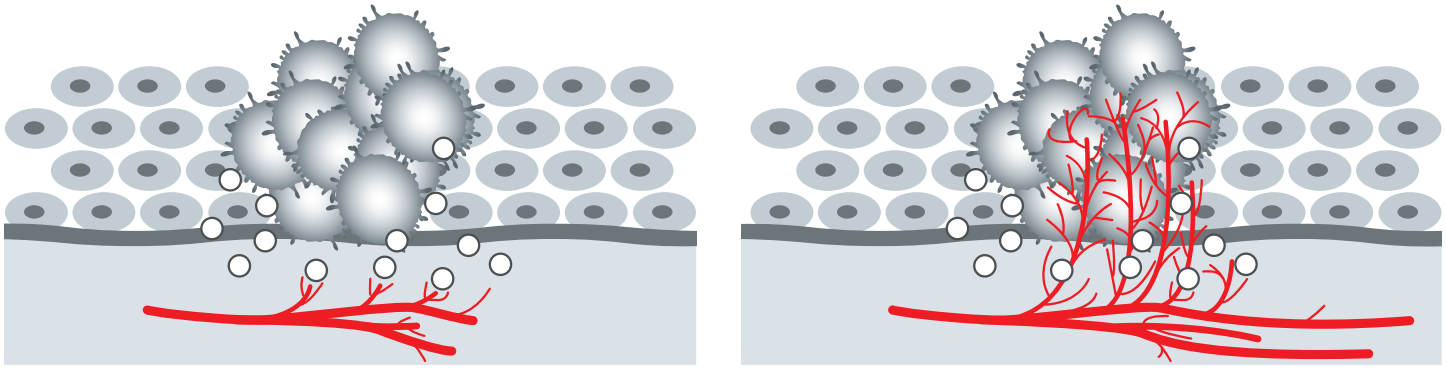


Figure 2: Tumor angiogenesis. *In order to grow and spread, a small localized tumor induces angiogenesis by secreting various proteins/growth factors (e.g. VEGF). Newly formed blood vessels provide a better supply with nutrients, oxygen and growth factors.*

The Angiogenesis Market

As the global incidence of cancer continues to rise, the market for anti-cancer therapies increases concomitantly. Angiogenesis inhibitors have potential as a unique cancer therapy – rather than attacking the cancerous cells, they target the pathological angiogenesis that allows the cancer to grow. As they are a new class of anti-cancer therapy, they have a great deal of potential. There are several anti-angiogenesis factors under review, including growth factors, receptor kinases, transcription factors and molecules in the MPK and PI3K signaling pathways.

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In healthy adults, blood vessels typically do not grow (angiogenesis does not occur) except during the menstrual cycle

♀

Human endometrium is angiogenic throughout the menstrual cycle

5

is the number of angiogenic growth factors tested in humans for wound healing and restoration of blood supply to heart, limbs and brain

1975

The first angiogenesis inhibitor molecules were discovered by Dr. Judah Folkman and Dr. Henry Brem in 1975

>300

Is the amount of angiogenesis inhibitors have now been discovered

>184

>184 million patients in the Western world would benefit from antiangiogenic therapy

>314

>314 million patients in the Western world would benefit from angiogenesis stimulating therapy

20

Is the number of known angiogenic growth factors

30

Is the number of known natural angiogenesis inhibitors found in the human body

Assaying Angiogenesis

Appropriate selection of an assay for angiogenesis is crucial to successful research, and depends on various factors including the scientific question being asked, the precise molecular mechanism being studied, and the final scientific or clinical goal.

For example, investigating the molecular mechanisms occurring during angiogenesis required an assay to resolve individual molecular steps accurately and reproducibly. If you wish to understand how a specific pro- or anti-angiogenic factor works, you would need an assay that replicates the angiogenic steps as seen under the physiological or pathological state being studied.

Tube Formation Assay

This assay allows measurement of the capacity endothelial cells have to form tubes (capillary-like structures), and therefore undergo angiogenesis. This assay is commonly used to determine if a compound inhibits or promotes angiogenesis, in addition it can determine:

- Which genes or pathways are involved
- What effect given factors have on tube formation
- How signals are transduced during angiogenesis
- The cytoskeletal effects of angiogenesis
- Identification of endothelial progenitors

Migration Assay

During angiogenesis, endothelial cells break down the basement membrane using proteolytic enzymes and migrate in response to a gradient of angiogenic factors (such as VEGF). Assays exist to assess this endothelial cell migration in response to angiogenesis inducing or inhibiting factors.

Invasion Assay

Assays are available to measure the capacity for endothelial cells to invade the basement membrane. Understanding the invasion process enables study of the mechanisms of angiogenesis in injured tissue and cancer.

Proliferation Assay

Proliferation of endothelial cells is needed to allow capillaries to develop. Whether factors are pro- or anti-angiogenic can be measured by direct cells counts, metabolic activity and DNA synthesis.

Other assays to study angiogenesis include the aortic ring/arch assays or the *in vivo* chorioallantoic membrane (CAM) and corneal angiogenesis assays.

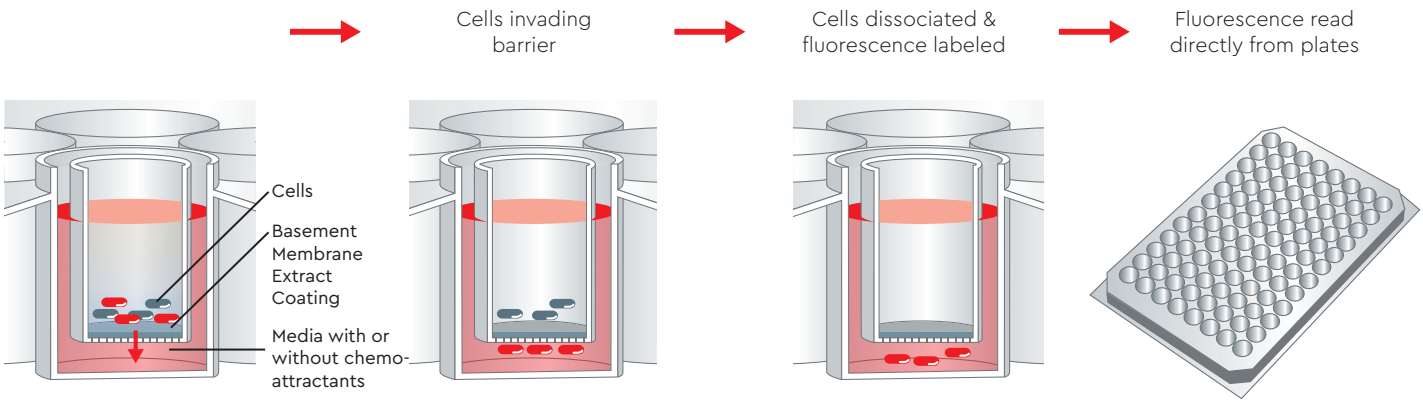

Figure3: *Cell invasion assay protocol using a Basement Membrane Extract-coated Boyden Chamber.*

Angiogenesis Stimuli

Angiogenesis is stimulated by proteins via signalling pathways, including:

- Vascular endothelial growth factor (VEGF)
- Platelet derived growth factor (PDGF)
- Basic fibroblast growth factor (bFGF)
- Transforming growth factor (TGF)
- Tumor necrosis factor (TNF)
- Epidermal growth factor (EGF)
- Hepatocyte growth factor (HGF)
- Granulocyte colony-stimulating factor (G-CSF)
- Placental growth factor (PlGF)
- Interleukin-8 (IL-8)
- Various other cytokines and enzymes

Hypoxia upregulates the expression of different genes which lead to increased expression of these agents.

Factors to Consider when Assaying Angiogenesis

- **Cell type**
  Tube-forming capacity varies between endothelial cell types, therefore assay conditions and cell types should closely mimic the disease or angiogenic conditions being studied.

- **Basement Membrane-Like Matrix**
  Different matrices cause different rates of differentiation, so choosing the right matrix is important. In tube formation assays, the basement membrane can be prepared from tissues or tumors, or a gel matrix layer of fibrin/fibronectin, laminin, collagen or BME/Matrigel.

The Future of Angiogenesis

As we have seen, angiogenesis is a complex and fascinating process. Fully understanding it could enable the development of further anti-angiogenic therapies, especially in cancer therapy. Restoring vascular homeostasis also holds great potential in the treatment of ischemic disease, and angiogenesis could even be used to develop engineered tissues. Assaying angiogenesis is crucial to researching it, and appropriate assay selection an important step.

About us

At PromoCell, we help scientists do better research with a world-class portfolio of human primary cells, stem cells, blood cells and optimized cell culture media. With over 30 years of expertise, we are recognized globally for supplying scientists with the tools and training they need to do groundbreaking research.

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References

1. Adair TH, Montani JP. Angiogenesis. San Rafael (CA): Morgan & Claypool Life Sciences; 2010.
2. Ahmed Z, Bicknell R. Angiogenic signalling pathways. Methods Mol Biol 2009;467:3–24.
3. Angiogenesis Foundation. Accessed at: https://angio.org/learn/angiogenesis/facts-figures/ Last accessed: 3-Sep-2021
4. Camare C, Pucelle M, Salvayre-Negre A, Salvayre R. Angiogenesis in the atherosclerotic plaque. Redox Biol 2017;18–34.
5. Cheng R, Ma JX. Angiogenesis in Diabetes and Obesity. Rev Endocr Metab Disord 2015;16(1):67–75.
6. DeCicco-Skinner KL, Henry GH, Cataisson C, et al. Endothelial cell tube formation assay for the *in vitro* study of angiogenesis. J Vis Exp 2014;91:51312.
7. Fried L, Arbiser JL. Application of Angiogenesis to Clinical Dermatology. Adv Dermatol 2008;24:89–103.
8. Huang Z, Shi-Deng B. Roles of main pro- and anti-angiogenic factors in tumor angiogenesis. World J Gastroenterol 2004; 10(4):463–470.
9. Humar R, Zimmerli L, Battegay E. Angiogenesis and hypertension: an update. Journal of Human Hypertension 2009;23:773–782.
10. Karamysheva AF. Mechanisms of angiogenesis. Biochemistry (Mosc) 2008;73(7):751–62.
11. Rajabi M, Mousa SA. The role of angiogenesis in cancer treatment. Biomedicines 2017;5(2):34.
12. Ribatti D, Puxeddu I, Crivellato E, Nico B, Vacca A, Levi-Schaffer F. Angiogenesis in asthma. Clinical and Experimental Allergy;39(12):1815–1821.
13. Saaristo A, Karpanen T, Alitalo K. Mechanisms of angiogenesis and their use in the inhibition of tumor growth and metastasis. Oncogene 2000;19:6122–6129.
14. Shaikh S, Pajankar S, Sumant O. Cancer Therapeutics Market 2021. Allied Market Research 2021.
15. Staton CA, Stribbling SM, Tazzyman S, Hughes R, Brown NJ, Lewis CE. Current methods for assaying angiogenesis *in vitro* and *in vivo*. Int J Exp Pathol 2004;85(5):233–248.
16. Stryker ZI, Rajabi M, Davis PJ, Mousa SA. Evaluation of angiogenesis assays. Biomedicines 2019;7(37).
17. Tahergorabi Z, Khazaei M. A review on angiogenesis and its assays. Iran J Basic Med Sci 2012;15(6):1110–1126.
18. Walsh DA, Pearson CI. Angiogenesis in the pathogenesis of inflammatory joint and lung diseases. Arthritis Res 2001;3(3):147–153.
19. Zirlik K, Duyster J. Anti-angiogenics: current situation and future perspectives. Oncology Research and Treatment 2018;41:166–171.

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