VascuNet™ Pericyte Co-Culture Assay

A stable, more physiological, in vitro angiogenesis model

- VascuNet tube networks approximate *in vivo* vessels more closely than other systems
- Allows for long-term *in vitro* analyses of pro- and anti-angiogenic compounds on established vessels
- PC-M, hESC-derived pericytes, resemble pericytes *in vivo*, functionally and phenotypically

A crucial interplay between cells

Angiogenesis is a central process in development, reproduction, and wound healing that requires tightly regulated interactions between cells; primarily, between endothelial cells and pericytes. Endothelial cells break down basement membranes, proliferate, migrate, and re-organize to form capillary structures. Pericytes do not appear to be necessary in the initial formation of the vascular network. However, they are essential to ensuring proper maturation and stability of blood vessels. Pericytes promote the differentiation and proliferation of endothelial cells and play a crucial role in driving the formation of vascular branches. Most notably, pericytes are integral to stabilizing vascular tubules and improving sprout integrity.

When a breakdown in communication occurs between pericytes and endothelial cells, the equilibrium balance is tipped causing increased or aberrant angiogenesis that can lead to numerous pathologies. Such is the case in diseases including macular degeneration, tumor progression, psoriasis, arthritis, stroke, and impaired wound healing. Interestingly, it is believed that in many cases the malfunction of pericytes is central to disease progression.

Although endothelial cells reorganize to form long lasting, three-dimensional vascular networks *in vivo*, most *in vitro* assays are limited in their utility because they lack requisite components, namely pericytes. Without these stabilizing cells, networks developed by HUVEC monocultures are usually maintained for only 24 hours. Other co-culture methods include unrelated cell types that may act as feeders or convey limited stabilizing functions, but are not physiologically relevant.

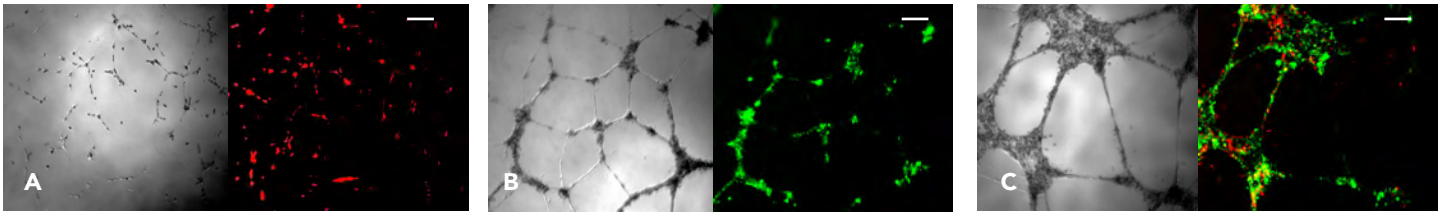

Figure 1. Mono- and co-cultures of HUVECs and Pericytes on Growth Factor Reduced Matrigel®. [A] Pericytes derived from hESCs (VascuNet PC-M cells) and seeded at 6000 cells/cm^2, do not form tubular networks in monoculture after 24 hours. PC-M cells were stained with Vybrant® DiI (orange-red). [B] Within 24 hours of culture, VascuNet HUVECs seeded at 120,000 cells/cm^2, form a network of tubules. HUVECs were stained with Vybrant DiO (green). [C] Co-culture of VascuNet HUVECs and PC-M cells seeded at a 20:1 ratio, exhibit an extensive network of tubule formation after 24 hours that is stable for up to 6 days (see Figure 2, top row). Scale bar represents 200µm, images taken at 4x magnification.

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A more clinically-relavant co-culture assay

The VascuNet Pericyte Co-Culture Assay is a novel angiogenesis model that includes HUVEC and PC-M cells to study the effects of drug and therapeutic compounds on vascularization in a 96-well plate. PC-M cells are derived from a proprietary differentiation method using the ESI-017 Human Embryonic Stem Cell Line. Cells and media components undergo strict quality screening to ensure reproducibility. Networks are maintained for up to four to six days, allowing for a more extensive and clinically-relevant model system for studying the effects of compounds on tube formation.

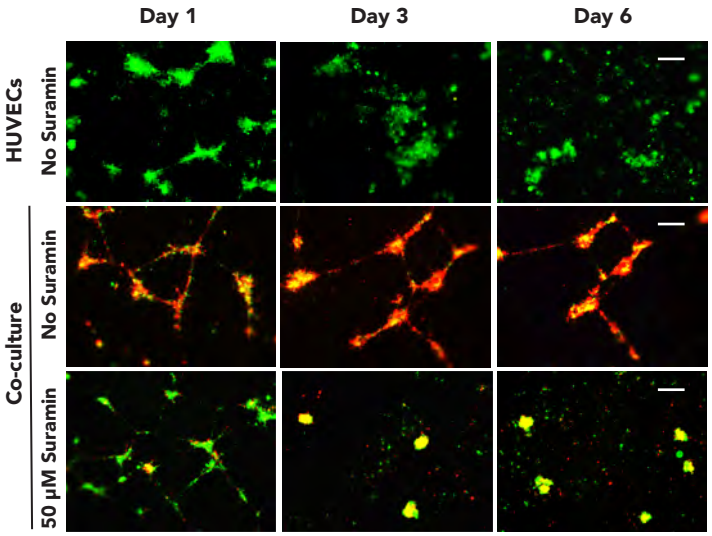

Figure 2. HUVEC monocultures (top row) seeded at 120,000 cells/cm^2 and stained with Vybrant DiO (green), reveal a stable structure 24 hours post seeding. By day 3, network structures are no longer visible. Co-culture of VascuNet HUVECs (green) and PC-M cells stained with Vybrant Dil (orange-red) (middle row) were seeded at 42,000 cells per well at a ratio of 20:1 HUVECs:PC-M cells. A vascular network is formed on day 1 and remains stabilized by the presence of PC-M cells at days 3 and 6 post seeding. The vascular network is diminished in the presence of the anti-angiogenic compound, Suramin (bottom row), added at day 1, and does not recover despite cell proliferation from days 3 to 6. All cultures grown on Growth Factor Reduced Matrigel. Scale bar represents 200µm, images taken at 4x magnification.

REFERENCES

Gerhardt, H. and Betsholtz, C. (2003) Endothelial-pericyte interactions in angiogenesis. Cell Tissue Res. 314: 15–23.

Hamilton, N.B., et al. (2010) Pericyte-mediated regulation of capillary diameter: a component of neurovascular coupling in health and disease. Front Neuroenergetics 2: 5.

Stratman, A.N., et al. (2009) Pericyte recruitment during vasculogenic tube assembly stimulates endothelial basement membrane matrix formation. Blood 114: 5091–5101.

von Tell, D., et al. (2006) Pericytes and vascular stability. Exp Cell Res. 312: 623–629.

ORDER INFORMATION

DESCRIPTION	SIZE	CAT. NO.
VascuNet™ Pericyte Co-Culture Assay	96 assays	EM2202

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