Human Endothelial Cell Spheroid-based Sprouting Angiogenesis Assay in Collagen

Commonly used acronym: *In vitro sprouting assay*

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Organisation

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Basic Research
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	HUVEC (Human Umbilical Vein Endothelial Cell) or HDLEC (Human Dermal Lymphatic Endothelial Cell)

DESCRIPTION

Method keywords

(lymph)angiogenesis
Endothelial cells
growth factor supplementation
spheroids

Scientific area keywords

vascular development
blood vessel
tumor stroma
sprouting angiogenesis

Method description

This assay evaluates the sprouting ability of endothelial cells in a collagen matrix and it used as an *in vitro* model to study the formation of new blood/lymphatic vessels. The effect of pro-angiogenic growth factors or co-cultured cells can be measured by

quantifying the amount of vascular sprouts that form on endothelial spheroids. Endothelial spheroids are obtained by growing endothelial cells in hanging drops, which forces the cells to adhere to each other. After generation of the spheroids, they are embedded in a collagen matrix in which endothelial growth factors or specific cell types can be embedded. Finally the amount of endothelial sprouts is quantified as a measure of the endothelial sprouting propensity.

Lab equipment

- Biosafety cabinet,
- CO2 incubator,
- Microscope.

Method status

History of use
Internally validated
Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

The method is simple and the vascular sprouts share multiple morphological characteristics of vascular tip cells *in vivo.*

Challenges

The model is limited to evaluating sprout propensity, which is only the first step in the angiogenic cascade. The subsequent steps of tubule and network formation cannot be evaluated.

Future & Other applications

This method could also be used using conditioned media from other cell types (for example cancer cells). As such, it could be used to asses the amount of (lymph)angiogenic signaling in the secreted medium.

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Meçe, O., Houbaert, D., Sassano, ML. et al. Lipid droplet degradation by autophagy connects mitochondria metabolism to Prox1-driven expression of lymphatic genes and lymphangiogenesis. Nat Commun 13, 2760 (2022). https://doi.org/10.1038/s41467-022-30490-6

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