

# 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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**Specific Research Group or Service** Laboratory of Cell Death Research & Therapy (VIB-KU Leuven)

**Country** Belgium

## SCOPE OF THE METHOD

<b>The Method relates to</b>	Human health
<b>The Method is situated in</b>	Basic Research
<b>Type of method</b>	In vitro - Ex vivo
<b>Specify the type of cells/tissues/organs</b>	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 is 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 assess 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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