NEUROMICS

3D Human HUVECs Angiogenesis

Catalog #: 3D45001

General Information

Our 3D Human HUVECs Angiogenesis model is constructed using GFP-Tagged human umbilical vein endothelial cells (HUVECs) are co-cultured with RFP-Tagged supporting cells. GFP positive capillary like tubule formation can be monitored in real time under fluorescence microscope throughout the whole process of the experiment.

The 3D Human HUVECs Angiogenesis contains the materials necessary to preform multiple angiogenesis assays in 6, 12, or 24 well formats. The 3D model is designed that the testing materials, i.e. compounds, conditioned media, or tissue explants, can be added into the system at any time, ranging from the onset of vasculogenesis to advanced angiogenesis. The resulting effect on tubule formation (tubular length, number of branches, etc.) can be monitored throughout the whole process under inverted fluorescence microscope.

Reagents and Materials Provided:

- 1x 24, 12 or 6 well plate of seeded tissue in the insert
- 1 x 500ml of Endo-Growth Medium (4°C).

The 3D Human HUVECs Angiogenesis Model is a proprietary system in which GFP-tagged human endothelial cells from variable vascular beds are co-cultured with RFP-tagged human supporting cells in a specially designed medium. The endothelial cells initially form small islands within the culture matrix. They subsequently begin to proliferate and then enter a migratory phase during which they move through the matrix to form threadlike tubule structures with lumens. They gradually join up (by 1 - 2 weeks) to form a network of anastomosing tubules, which closely resembles the capillary bed found in vivo.

Protocol

Day 1:

1. Pre warm Endo-Growth Medium to 37ºC in a water bath
2. Accurately pipette 65ml Endo-Growth Medium into a Falcon tube
3. Thaw plate in a (37ºC, 5% CO2 and humidified incubator)
4. Once thawed quickly change the media
5. Add 300 microliters of fresh media inside each of the inserts.
6. Add 1ml to the each of the wells, make sure the media touches the bottom of the insert.
7. Place the plate in an incubator (37ºC, 5% CO2 and humidified) for 2-3 hours.
8. After 2-3 hours in the incubator, remove the media from the plate and the inserts
9. Repeat steps 5 and 6.
10. Place the plate in an incubator (37ºC, 5% CO2 and humidified

Day 2:

- Take the plate from the incubator and examine cells under inverted fluorescence microscopy (GFP positive HUVECs should sparsely and evenly distributed among RFP positive human supporting cells)
- Wash the cells one with 2 ml of PBS

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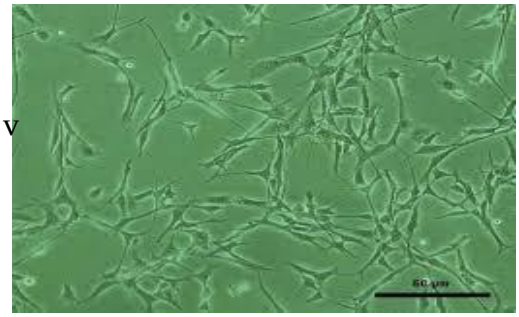
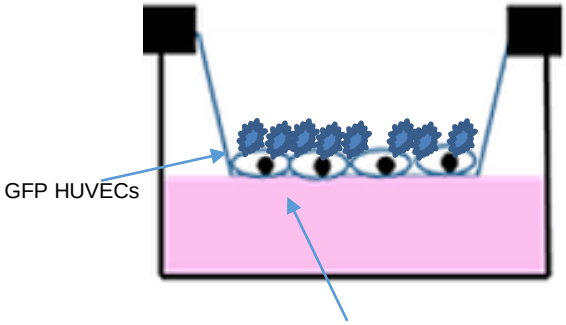
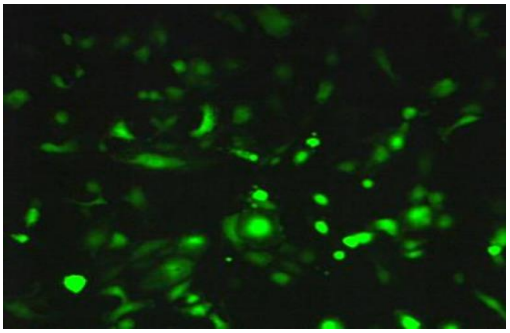
- Add 2.0ml of fresh Endo-Growth medium (control) or Experimental media (Endo-Growth medium, plus pro- or anti-angiogenic regents according to customer's needs)
- Place the plate back into the incubator. Day 4, 6, 8, 10, 12, and 14…… 13. Replace the medium every 2 days until the end of the experiments.
- Tube formation and cellular networks will start about day 7 or sooner.

V

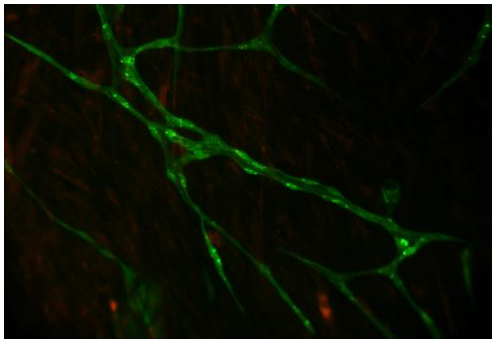


HUVECs growing in inserts

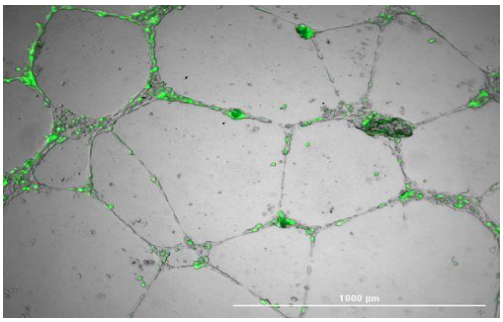




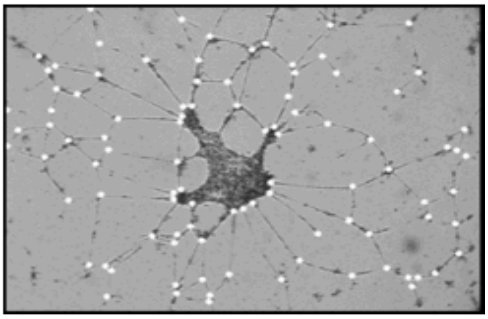
HUVECs before tube formation



HUVECs after tube formation



4x overlay image of HUVECs used in an endothelial cell capillary tube formation assay. The cells were stained with calcein. Imaging was performed in the brightfield and GFP channels



Co-culture system

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