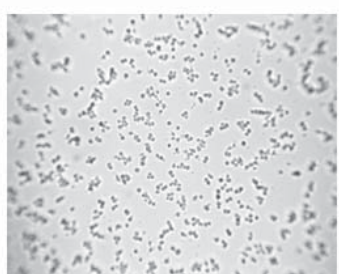


Factors Affecting Basement Membrane Protein Dependent in vitro Cell Models

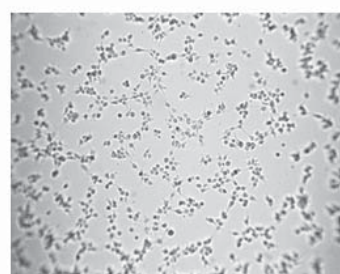
TUBE FORMATION ASSAYS

Dynamics of Tube Formation

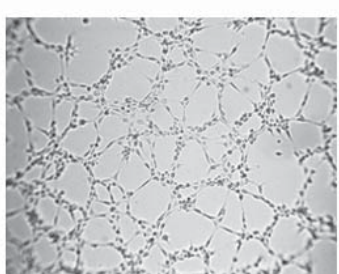
Attachment and alignment
1 hour incubation



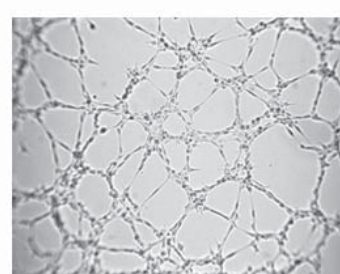
Elongation and migration
2 hours incubation



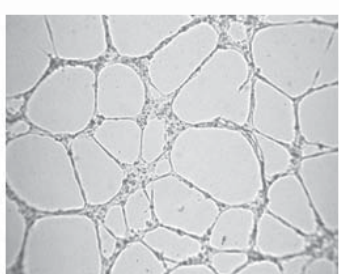
Formation of polygonal network
4 hours incubation



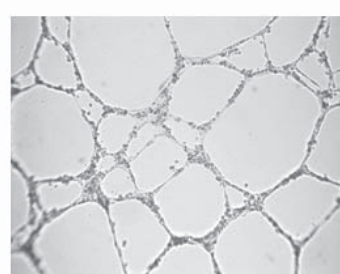
Stabilized "honeycomb"
6 hours incubation



Network with lumen
16 hours incubation



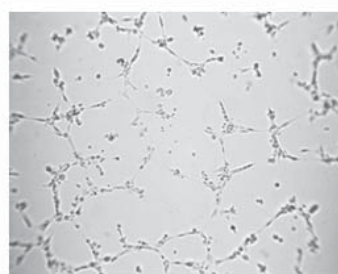
Degradation and detachment
24 hours incubation



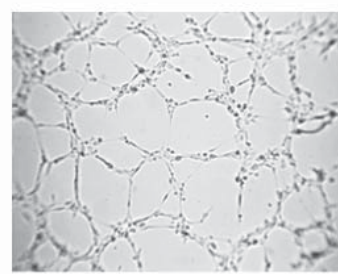
Human Umbilical Vein Endothelial Cells (HUVEC) were seeded on RGF BME gels at 46,870 cells/cm² (15,000 cells/well of a 96-well plate) and incubated in the Endothelial Growth Medium-2 (EGM-2).

Passage Number After Thawing Affects Tube Formation

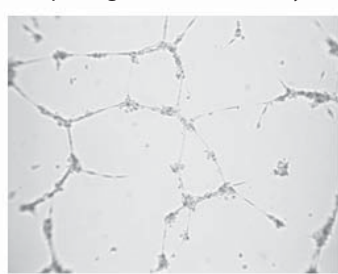
passage 0; 3 hours assay



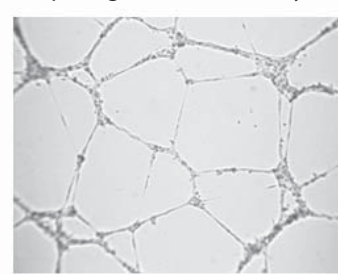
passage 2; 3 hours assay



passage 0; 20 hours assay



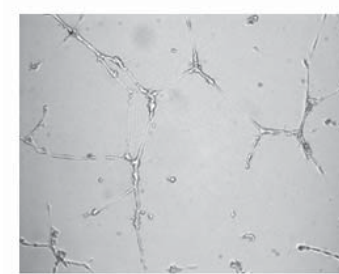
passage 2; 20 hours assay



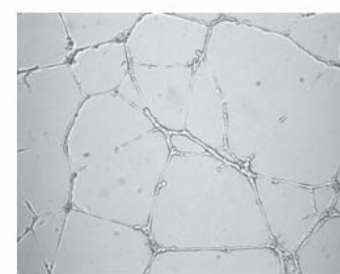
Human Umbilical Vein Endothelial Cells (HUVEC) at passages 0 and 2 were plated on RGF BME gels at 46,870 cells/cm² (15,000 cells/well of a 96-well plate) and incubated for 3 and 20 hours in the Endothelial Growth Medium-2 (EGM-2). NOTE: Use at least passage 2 cells for optimal tube assay results.

Cell Seeding Density Affects Formation of Tubular-like Network

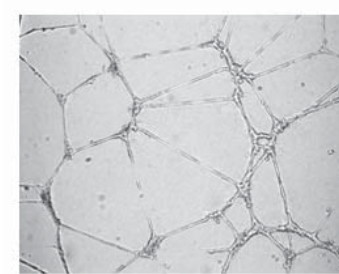
15,600 cells/cm²



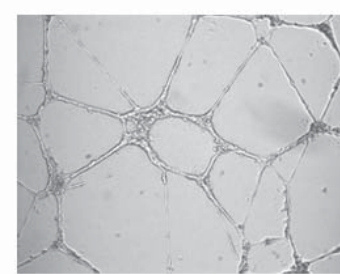
31,250 cells/cm²



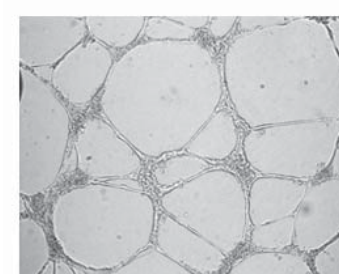
46,870 cells/cm²



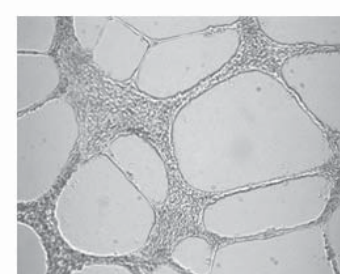
62,500 cells/cm²



93,750 cells/cm²



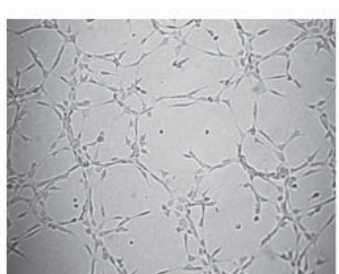
125,000 cells/cm²



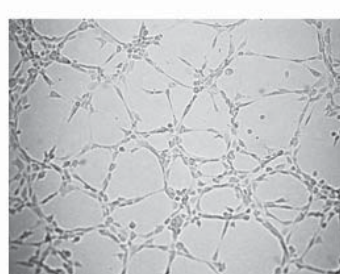
Human Umbilical Vein Endothelial Cells (HUVEC) were seeded on RGF BME gels at various concentrations and incubated for 16 hours in Endothelial Growth Medium-2 (EGM-2). NOTE: Cell density between 46,870 and 62,500 cells/cm² is optimal.

Tube Formation on Normal BME and Reduced Growth Factors (RGF) BME

Normal BME-basal medium



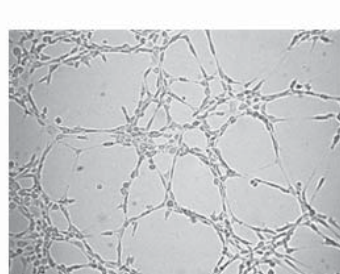
Normal BME-complete medium



RGF BME-basal medium



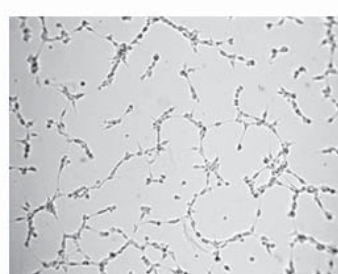
RGF BME-complete medium



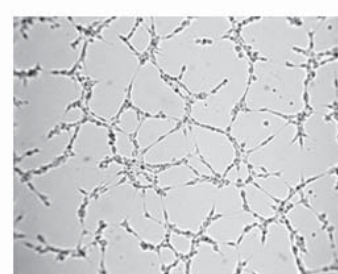
SVEC4-10 cells were seeded onto RGF BME gels or Normal BME gels at 46,870 cells/cm² on a 96-well plate and incubated for 3 hours in the basal medium (EBM-2) or in the complete growth medium (EGM-2). NOTE: Tube formation requires angiogenesis stimulators (growth factors). The background tube formation is lower with RGF BME.

Induction of Tube Formation by Growth Factors and FBS

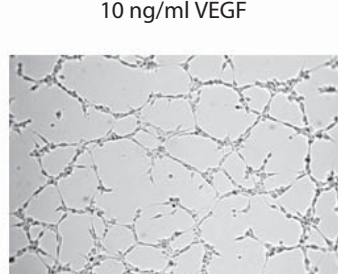
EBM-2 (basal medium)



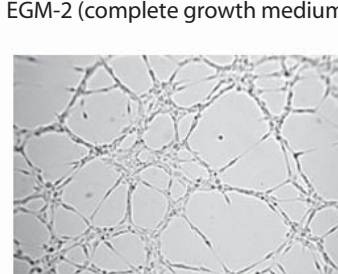
EBM-2 + 35 ng/ml bFGF



EBM-2 + 35 ng/ml bFGF
10 ng/ml VEGF



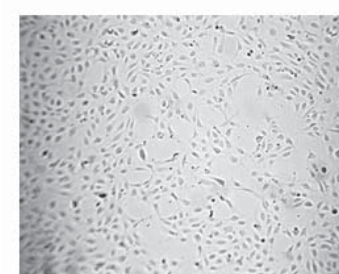
EGM-2 (complete growth medium)



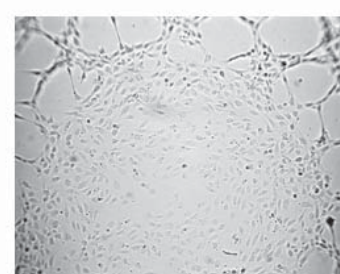
HUVECs were seeded on RGF BME gels (46,870 cells/cm²) and incubated for 6 hours in the Endothelial Basal Medium-2 (EBM-2) with and without FGF-2 and VEGF or in the Endothelial Growth Medium-2 containing 2% FBS, FGF-2, VEGF and other supplements (EGM-2). NOTE: The best tubes are with a combination of all the factors and serum.

Endothelial Cells do not Form Tubular-like Network on Thin BME Gels

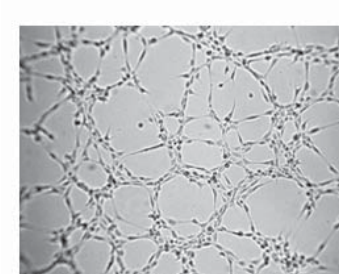
10 µl BME per well



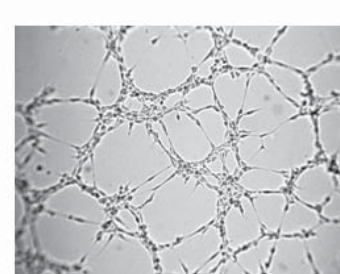
25 µl BME per well



50 µl BME per well



100 µl BME per well



HUVECs were seeded onto RGF BME gels (46,870 cells/cm²) on a 96-well plate and incubated for 6 hours in the Endothelial Growth Medium-2 (EGM-2). NOTE: At least 50 µl BME/well of a 96-well plate required for optimal tube formation.

ANGIOGENESIS

PRODUCT NAME

Cultrex[®] In Vitro Angiogenesis Assay Kit – Tube Formation
Cultrex[®] DIVAA™ Starter Kit
Cultrex[®] DIVAA™ Activation Kit
Cultrex[®] DIVAA™ Inhibition Kit
Cultrex[®] In Vitro Angiogenesis Assay Endothelial Cell Invasion

CAT

3470-096-K
3450-048-SK
3450-048-K
3450-048-1K
3471-096-K

BASEMENT MEMBRANE EXTRACT

PRODUCT NAME

Cultrex[®] BME with Phenol Red
Cultrex[®] BME without Phenol Red
Cultrex[®] BME reduced growth factor with Phenol Red
Cultrex[®] BME reduced growth factor without Phenol Red
Cultrex[®] PathClear[®] BME with Phenol Red
Cultrex[®] PathClear[®] BME without Phenol Red
Cultrex[®] PathClear[®] reduced growth factor BME with Phenol Red
Cultrex[®] PathClear[®] reduced growth factor BME without Phenol Red
Cultrex[®] PathClear[®] HC20+™ BME (High Protein concentration BME)

CAT

3430-005-01
3432-005-01
3431-005-01
3433-005-01
3430-005-02
3432-005-02
3431-005-02
3433-005-02
3444-005-02

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