Assay Protocol

Endothelial Tube Formation Assay

gelomics
3D cell culture technologies

Introduction

Angiogenesis and vasculogenesis are processes underlying the formation of new blood vessels and involve the migration, growth, and differentiation of endothelial and supporting cell types, such as pericytes. Angiogenesis plays a critical role in the initiation and progression of diseases such as many cancers, autoimmune diseases, rheumatoid arhritis, and others. *In vitro* endothelial tube formation assays are particularly useful models for studying and modulating processes underlying the formation of new blood vessels to develop novel therapeutic approaches that may limit the progression of aforementioned diseases.

In the following procedure, we describe optimized assay conditions for the formation of endothelial tubes using a co-culture of human umbilical vein endothelial cells (HUVECs) and pericytes using LunaGel™ Photocrosslinkable Extracullar Matrix as a 3D cell culture substrate.

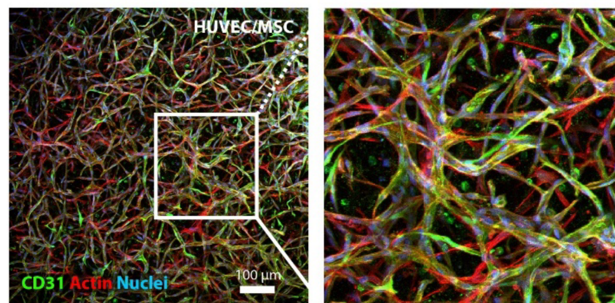

Figure: Capillary-like network formation of HUVECs and MSCs in LunaGel™ Photocrosslinkable Extracellular Matrix

HUVECs and MSCs were cultured in LunaGel™ (Porcine Skin Gelatin, 0.8 kPa) in the presence of VEGF, SDF-1, and FGF-2, fixed with 4% PFA, and stained for endothelial cell marker CD31 (green) and actin (red).

Materials

- Gelomics® LunaGel™ Photocrosslinkable Extracellular Matrix, Porcine Skin Gelatin, Low Stiffness (Gelomics Cat. No. SKU0002)
- 24 well flat-bottom non-treated tissue culture well plate
- Human umbelical vein endothelial cells (available from various vendors) cultured to 70-80% confluency. For best results, use cells below passage number of 8.
- Pericytes or mesenchymal stromal cells (MSCs) (available from various vendors) cultured to 70-80% confluency.
- Endothelial cell culture growth medium with supplements, e.g. EGM™-2 Endothial Cell Growth Medium-2 Bullet Kit (Available from Lonza Bioscience, Cat. No. CC-3162)
- Growth factors (available from various vendors). Add 50 ng/ml of complete EGM™-2 media, fresh at every media change
  - Stromal cell-derived factor-1 (SDF-1)
  - Fibroblast growth factor 2 (FGF-2)
  - Vascular endothelial growth factor

Procedure

The protocol below outlines the procedures for a final volume of 1 mL of cell laden LunaGel™ ECM. Adjust volumes as required based on your desired sample volume (30 µl recommended) and replicate number.

1. Place the 2X LunaGel™ ECM solution into a water bath at 37 °C for < 15 min.
2. Reconstitute one vial of the photoinitiator with 1 ml sterile PBS and store protected from light.
3. Lift and count cells according to your standard protocol. Inhibit and remove trypsin/protease solution from the cells.
4. For each 1 ml of LunaGel™ Photocrosslinkable Extracellular Matrix, transfer 6×10^6 HUVECs and 6×10^5 pericytes/MSCs into a single reaction tube (both cell types mixed into one tube) and centrifuge to

pellet cells. Remove the entire supernatant, taking care to minimize liquid residues that may dilute the ECM solution in later steps.

5. Add 500 µl of the LunaGel™ ECM solution to the cell pellet and gently pipette up and down to homogenously resuspend cells.
6. Add 500 µl of the photoinitiator solution to the cell suspension. Store the remaining photoinitiator solution at 4 – 8 °C, protected from light, for future use.
7. Mix thoroughly by pipetting up and down to ensure a homogenous cell suspension. Take care to avoid the introduction of air bubbles.
8. Dispense 30 µl of the LunaGel ECM™/cell suspension into the centre of each well of a 24 well plate to create dome-shaped samples.

   Tip: Use reverse pipetting to avoid introducing air bubbles when dispensing the mixture into well plates.
9. Carefully transfer the well plate to the LunaCrosslinker, taking care to avoid any disturbance/smudging of the samples before crosslinking.
10. Crosslink the cell laden LunaGel™ ECM by light exposure using the Luna Crosslinker™ for 50 seconds (~ 1.5 kPa elastic modulus).
11. Add 1 ml pre-warmed endothelial cell culture medium with growth factors to each well and incubate in a humidified cell culture incubator at 37 °C and 5% CO_2. Change 70% of the cell culture media every 2 days.

Expected results and downstream assays

Capillary-like structures and highly connected cellular networks should form within 2-3 days of culture and mature into lumen-containing tubules over time. These structures can be observed using brightfield microscopy.

Recommended downstream assays include:

- Live/Dead viability staining using fluorescein diacetate and propidium iodide
- Immunofluorescence analysis (for example CD31 endothelial marker)
- Gene expression analyis

Troubleshooting guide

Problem	Solution
LunaGel™ ECM solution solidifies during protocol	Re-heat LunaGel™ ECM solution (with or without cells) to 37 °C in a water bath. Once liquid, mix by pipetting up and down. Do not heat above 37 °C.
Air bubbles in LunaGel™ ECM solution.	Centrifuge solution (with or without cells) at 300*g* for 1 min and mix by pipetting up and down.
The LunaGel™ ECM solution does not crosslink when exposed to light.	Ensure photoinitiator solution is prepared fresh and/or extend crosslinking time.
Capillary structures not forming	Ensure healthy cells, fresh media and growth factors are used.
LunaGel™ ECM samples dissolve following cell encapsulation.	Traces of trypsin or other proteases may degrade the hydrogel samples. Ensure complete removal of trypsin before cell encapsulation by washing the cells pellet with medium of buffer.

Please contact us at info@gelomics.com for questions or more information.