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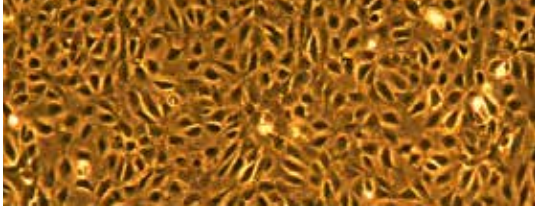
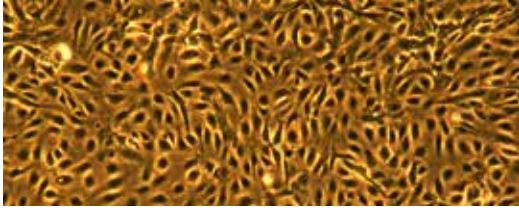




CELLMATRIX BASEMENT MEMBRANE GEL ATCC® No. ACS-3035

AP Note
No. 4

CELLMATRIX BASEMENT MEMBRANE GEL SUPPORTS *IN VITRO* ANGIOGENESIS ASSAYS

Abstract

This study will test the ability of CellMatrix Basement Membrane Gel to support *in vitro* angiogenesis assays.

Introduction

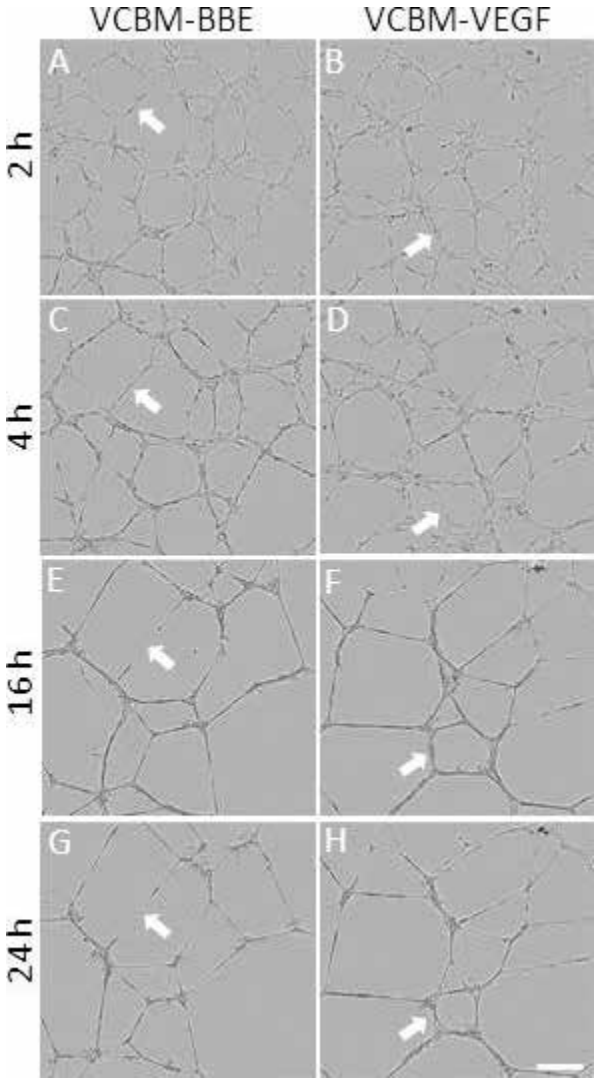
Angiogenesis can be examined *in vitro* by observing the differentiation of endothelial cells into tubule structures in a basement membrane gel[1]. Here, we tested the capability of CellMatrix, a basement membrane gel derived from the Engelbreth-Holm-Swarm (EHS) tumor cell line[2], to induce tubule formation in primary normal human umbilical vein endothelial cells (HUVECs). We cultured primary normal HUVECs in Vascular Cell Basal Medium (VCBM) supplemented with either Bovine Brain Extract (BBE) or Vascular Endothelial Growth Factor (VEGF) and monitored tubule formation for 36 hours (h).

Materials and Methods

Primary normal HUVECs (ATCC® PCS-100-010) were cultured in VCBM (ATCC® No. PCS-100-030) supplemented with either BBE (ATCC® No. PCS-100-040)[3] or VEGF (ATCC® No. PCS-100-041)[4] endothelial cell growth kits. A 24-well plate was coated with 200 µL/well of undiluted CellMatrix Basement Membrane Gel (ATCC® No. ACS-3035) and allowed to gel at 37°C for 30 min before the cells were seeded. HUVECs were dissociated and re-suspended in growth media, and seeded onto CellMatrix at 4 x 10^4 cells/cm^2. Tubule formation was monitored over time using phase contrast microscopy in an IncuCyte™ FLR (Essen BioScience, Ann Arbor, MI) with images acquired every 2 h for 36 h.

Results and Discussion

ATCC primary HUVECs cultured in VCBM-BBE began to form tubules 2 h post seeding on CellMatrix (A, arrow), while the tubules in the VCBM-VEGF media were still relatively undeveloped (B, arrow). By 4 h post seeding, tubules were more advanced in the VCBM-BBE media compared to the tubules in the VCBM-VEGF media, but tubules in the VCBM-VEGF had formed at a greater density than in the VCBM-BBE media (compare C and D). The tubules cultured in VCBM-BBE formed faster than in the VCBM-VEGF media, but they also began to deteriorate earlier (at 16 h) than the tubules formed in the presence of VEGF (compare E and F, arrows). By 24 h, the tubules, in both media formulations, had begun to deteriorate (G, H, arrows).



Primary normal HUVECs develop tubules when cultured on CellMatrix™ basement membrane gel. Scale bar = 200 µm

Therefore, we find that CellMatrix Basement Membrane Gel is able to support angiogenesis of HUVECs cultured in VCBM supplemented with either BBE or VEGF Endothelial Cell Growth Kits. HUVECs form more stable tubules when VEGF growth kit supplements are added to the media. In contrast, when BBE growth kit supplements are added to the media, the HUVECs form tubules more rapidly, but these tubules are less stable and degrade earlier.

Conclusion

CellMatrix Basement Membrane Gel provides a suitable platform for *in vitro* angiogenesis assays and allows for the observation of subtle differences in tubule growth under different culture conditions.

References

1. Arnaoutova I, George J, Kleinman HK, Benton G. The endothelial cell tube formation assay on basement membrane turns 20: state of the science and the art. *Angiogenesis*. (2009);12(3):267-74.
2. ATCC Product Information Sheet: ACS-3035 CellMatrix Basement Membrane Gel.
3. ATCC Product Information Sheet: PCS-100-040 Endothelial Cell Growth Kit-BBE.
4. ATCC Product Information Sheet: PCS-100-041 Endothelial Cell Growth Kit-VEGF.

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