

## **Human Primary Lung Endothelial Cells in Culture**

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ONLINE DATA SUPPLEMENT

## **Real Time Movie of In Vitro Tube Formation**

In vitro tube formation was assessed using a Matrigel endothelial cell tube formation assay (Chemicon International, Temecula, CA) (Yahata, Shirakata et al. 2003). Matrigel, a solid gel of basement membrane proteins, prepared from Engelbreth-Holm-Swarm mouse tumor was added to 6 well plate and allowed to gel for >1 h at 37C. PAEC were seeded on the surface plates at  $18 \times 10^4$  cells/well. Angiogenesis was observed by phase contrast microscopy and documented by collecting digital images over time (0 to 8 h).

The time course of angiogenesis was evaluated by collecting and quantitating time-lapse movies of cells, using a live-cell imaging system and Metamorph software (Universal Imaging, Downingtown, PA).