

## Dynamic and Complex Cell Movement during Formation of Vascular Structures with the Fibrin Bead Assay

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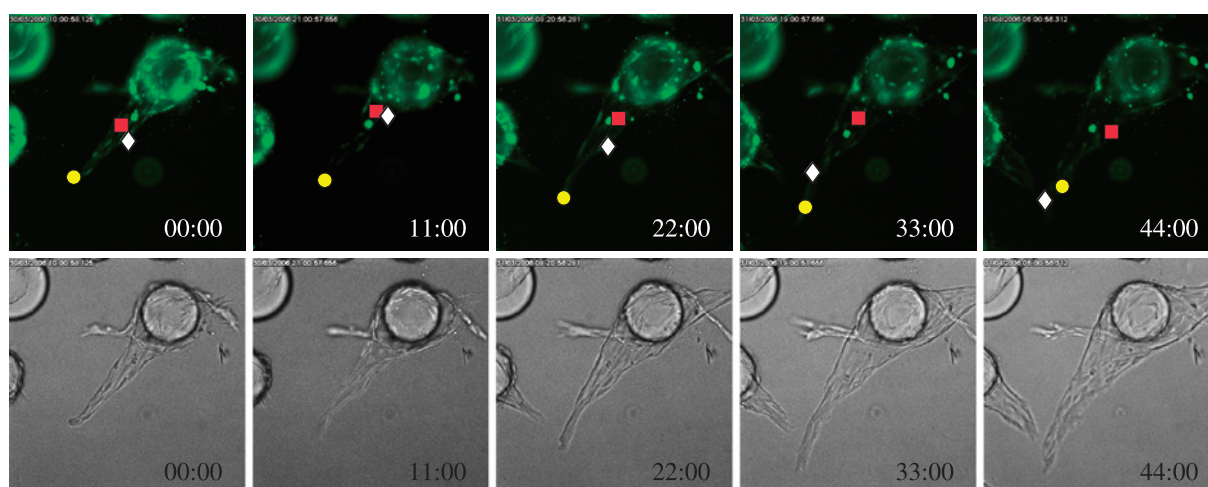


Fig. 1

Blood vessels begin to form from sprouts of endothelial cells, and these projections determine various morphologic features of the vasculature. Cell movements are a primary determinant of vascular patterning during blood-vessel formation. In this photogravure, endothelial cell motility was shown at the single-cell level, and dynamic and complex cell selectivity in direction and in migration distance was demonstrated *in vitro*.

**Fig. 1** Images of live human umbilical vein endothelial cells during vessel growth with the fibrin gel assay. Human umbilical vein endothelial cells (HUVECs) labeled with Vybrant carboxyfluorescein diacetate, succinimidyl ester (CFDA SE) (Molecular Probes) were monitored with Leica AS MDW time-lapse video microscopy. Fluorescent (upper) and bright-field (bottom) images indicate the growth of vascular sprouts every 11 hours. The yellow circle, white diamond, and red square show the positions of each cell. Growth direction of the vascular sprout was not completely consistent with the motility of HUVECs. Some cells moved forward whereas some cells moved backward during vascular protrusion.

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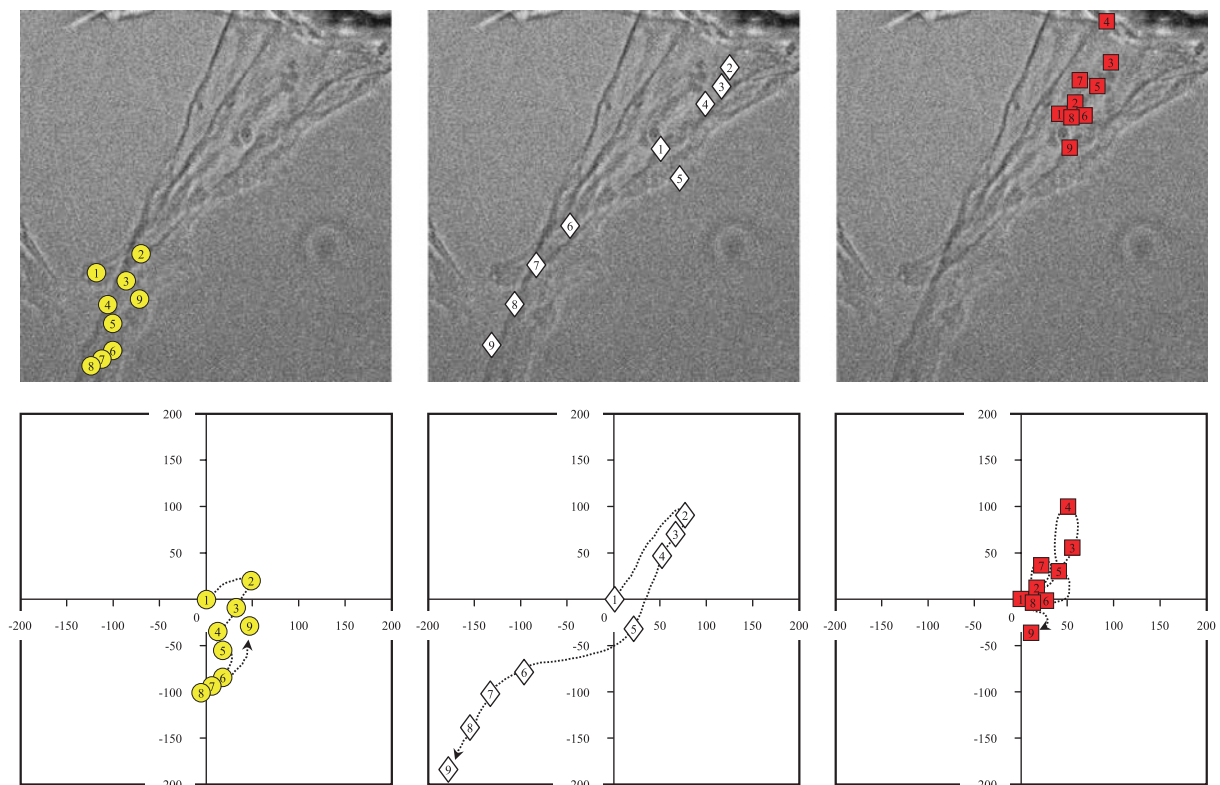


Fig. 2

**Fig. 2** Complex HUVECs arrangements during the formation of vessel-like structures. Positions of HUVECs analyzed with a Leica AS MDW microscope were plotted every 5.5 hours. The numbers of each mark show the order of cell positions over 44 hours. The lower panels show the direction and distance moved every 5.5 hours from the starting point ( $x=0, y=0$ ). Three arbitrarily chosen cells each displayed distinct trajectory patterns. Migration speed was not constant within each cell and differences in the three trajectories were apparent. These results indicate the dynamic and random movements of HUVECs during vascular formation.