**Abstract**

Angiogenesis is a multi-step, complex process regulated by growth factors, enzymes, and extracellular matrix molecules. In vivo, the angiogenic process involves multiple cell types interacting in concert to cause proliferation, migration, and differentiation of endothelial cells into micro-vascular tubular arrays. Angiogenesis inhibitors targeting vascular growth factors, signaling pathways and matrix proteases are demonstrably effective in both preclinical models and clinical use, but the effectiveness of this form of therapy can be transitory due to evasive and intrinsic resistance. The discovery of new classes of anti-angiogenic drugs has proven difficult, as few in vitro systems adequately model the entire process. Here, using a compact fluorescent imaging instrument with a kinetic co-culture model of angiogenesis, involving GFP-infected HUVECs and normal human fibroblasts, we demonstrate the ability to visualize and quantify all stages of in vitro angiogenesis in a time-dependent manner for up to 14 days. Algorithms were developed to easily quantify tube length, area, and branching to measure the effects of pro-angiogenic and anti-angiogenic agents. Further, we examined the contribution of endogenous VEGF on tube formation using anti-VEGF antibodies. As expected, the effects of all pharmacological agents tested were time- and concentration-dependent. Some relations were monophasic, whereas others more complex. Ongoing studies are designed to develop in vitro models of resistance to anti-angiogenic drugs by using combinations of pro-angiogenic factors that more closely mimic the condition found in vivo.

**IncuCyte Technology**

- Fits in an incubator; HD Phase optics
- LED light source; 50,000 hrs; relatively little heat
- Fluorescent optics optimized for measuring GFP in media
- Automated data acquisition and analysis
- Export images and movies

**Drug Effects on HUVEC Migration**

Fig. 2. A–D, IncuCyte/FLR angiogenesis images at days 1, 5, 9, and 14 of the assay, respectively. Scale bar: 300 μM E-H. Computational analysis of tube formation using the Angiogenesis Analysis Module. 1, 1j. Graphical analysis of tube length using media optimized for high (1) or low (J) endogenous tube formation.

**Summary and Conclusions**

Summary:
- IncuCyte™ FLR quantitatively assessed the time- and concentration-dependence of pharmacological agents in GFP co-culture and cell migration assays.
- bFGF and EGF potentiated VEGF-stimulated tube formation.
- Neutralizing VEGF antibodies inhibited bFGF and EGF effects.
- DLL4 selectively inhibited branching of tubules and the effect was reversed with small molecule inhibitors.
- VEGF, EGF, and DLL4 enhanced the rate of VEGF-mediated wound closure.

Conclusions:
- The GFP co-culture angiogenesis assay provides the means of quantitating the temporal effects of pharmacological agents on microvascular tubule formation to gain an in-depth understanding of their mechanism of action.
- Downstream assays such as cell migration can assist in decoding the full spectrum of the biological activity of these agents.

**Fig. 3. A–C**, Treatment with three growth factors (VEGF, FGF, and EGF) potentiate tube formation in a concentration-dependent manner. Representative images indicate growth factor potentiation (right) over control (left). Scale bar is 400 μM. D–F, Anti-VEGF treatment inhibits growth factor potentiation, as indicated in representative images below each graph. Anti-VEGF treatment in the presence of respective growth factor is shown on the right of each image set.