Modeling Angiogenesis in vitro: Comparing Two Human Co-Culture Approaches

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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 acting in concert to cause endothelial cell proliferation, migration, differentiation, and, ultimately, micro-vascular arrays. Under pathogenic conditions, changes in the micro-environment stimulate new vessel production. Anti-angiogenic therapies have shown promise at slowing disease, such as solid tumors, yet have proven transitory due to either inherent or acquired resistance. Advancement of in vitro angiogenesis models to study drug resistance and more complex pharmacologic paradigms, i.e., combination regimens, are paramount to developing the next generation therapies. Here, we investigate two different in vitro models of angiogenesis. The first model uses co-culture of human umbilical vein endothelial cells (HUVEC) with normal human dermal fibroblasts (NHDF) and the other uses co-culture of endothelial colony forming cells (ECFC) with adipose-derived mesenchymal stem cells (ADSC). These two models differ in several aspects, including the common cell components and growth factors used, but both allow for basic angiogenic networks, which are critical for drug testing. Here, we describe the development of these models and present the results of an assay to compare the performance of these two model systems.

Automated Imaging and Quantitation

Endothelial Cell Proliferation Differences

Combination Regimen Overcomes Resistance

Co-culture Models

Expression of Vascular Markers

Assay Highlights:
1. Imaging in fluorescence:
   - Continuous monitoring of tube formation via GFP fluorescence.
   - User control of drug treatment timing.
   - Quantiﬁcation of drug effects while experiment is ongoing.
2. Woundwell format allows for increased throughput:
   - 1-6 microwells in a single high-Well plate.
   - Acquisition/Analysis is automated.
3. Tiled Field of View acquisition modes images +/-50% of well.

Assay formats facilitate complex in vitro angiogenic experimentation:
1. Neangiogenic model – Investigate ability of test to augment or inhibit growth factor-mediated vasculogenesis.
2. Established models – Investigate ability of test agent to induce tube regeneration on established angiogenic networks.

HUVEC/NHDF

- Human model of HUVEC and NHDF that reflects all phases of angiogenesis process.
- Tube-like structures develop over 8-12 days.
- Initial proliferation and migration of HUVEC
- Differentiation of HUVEC into tubes
- Anastomosis and refinement to develop large angiogenic networks.

ECFC/ADSC

- Co-culture of mesenchymal stem cells derived from adipose tissue (ADSC) and endothelial colony forming cells (ECFC) derived from cord blood.
- Stimulation with VEGF promotes tube-like structure formation over 3-5 days.
- Initial differentiation and extension of ECFC;
- Connections between ECFC form smaller networks.
- Further refinement and Anastomosis defining larger angiogenic networks.

Figure 1: Software modules and different sample preparations in the Angiogenesis module.

Figure 2: Expression of angiogenic markers in both the HUVEC/NHDF and ECFC/ADSC models. A) Shows the successful expression of angiogenic markers (α-SMA, VEGF, and PDGFRα) in the HUVEC/NHDF model. B) Shows the successful expression of angiogenic markers (α-SMA, VEGF, and PDGFRα) in the ECFC/ADSC model. (Scale bars, 200µm).

Figure 3: Expression of angiogenic markers in both the HUVEC/NHDF and ECFC/ADSC models. A) Shows the successful expression of angiogenic markers (α-SMA, VEGF, and PDGFRα) in the HUVEC/NHDF model. B) Shows the successful expression of angiogenic markers (α-SMA, VEGF, and PDGFRα) in the ECFC/ADSC model. (Scale bars, 200µm).

Figure 4: In vivo angiogenic models to investigate vascular disruption. A) Treatment with an anti-VEGF antibody induces vascular regression in the Matrigel plug assay. B) Treatment with an anti-α-SMA antibody inhibits neovascularization in the Matrigel plug assay. C) Treatment with an anti-PDGFRα antibody inhibits neovascularization in the Matrigel plug assay. D) Treatment with an anti-Tie-2 antibody inhibits neovascularization in the Matrigel plug assay. E) Treatment with an anti-VEGF antibody inhibits neovascularization in the Matrigel plug assay. (Scale bars, 200µm).

Summary of in vitro Models

Table

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<tr>
<th>Cell Types</th>
<th>HUVEC/NHDF</th>
<th>ECFC/ADSC</th>
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<tr>
<td>Assay Duration</td>
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<tr>
<td>PDGFRα</td>
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<tr>
<td>Summary</td>
<td>Summary of 10 assays</td>
<td>Summary of 10 assays</td>
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<td>2. Mouse VEGF (monoclonal)</td>
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<td>3. Mouse PDGFRα (monoclonal)</td>
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<td>6. Mouse PDGFRα (polyclonal)</td>
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Ang-2 Signaling Alters Angiogenesis

Figure 5: Expression of angiogenic markers in both the HUVEC/NHDF and ECFC/ADSC models. A) Shows the successful expression of angiogenic markers (α-SMA, VEGF, and PDGFRα) in the HUVEC/NHDF model. B) Shows the successful expression of angiogenic markers (α-SMA, VEGF, and PDGFRα) in the ECFC/ADSC model. (Scale bars, 200µm).

Figure 6: In vivo angiogenic models to investigate vascular disruption. A) Treatment with an anti-VEGF antibody induces vascular regression in the Matrigel plug assay. B) Treatment with an anti-α-SMA antibody inhibits neovascularization in the Matrigel plug assay. C) Treatment with an anti-PDGFRα antibody inhibits neovascularization in the Matrigel plug assay. D) Treatment with an anti-Tie-2 antibody inhibits neovascularization in the Matrigel plug assay. E) Treatment with an anti-VEGF antibody inhibits neovascularization in the Matrigel plug assay. (Scale bars, 200µm).

Figure 7: Summary of in vitro models and drug treatment responses. A) HUVEC/NHDF co-culture model with Sunitinib (100 nM) and VEGF (100 ng/mL) treatment. B) ECFC/ADSC model with Sunitinib (100 nM) and VEGF (100 ng/mL) treatment. (Scale bars, 200µm).

Figure 8: Expression of angiogenic markers in both the HUVEC/NHDF and ECFC/ADSC models. A) Shows the successful expression of angiogenic markers (α-SMA, VEGF, and PDGFRα) in the HUVEC/NHDF model. B) Shows the successful expression of angiogenic markers (α-SMA, VEGF, and PDGFRα) in the ECFC/ADSC model. (Scale bars, 200µm).

Figure 9: Summary of in vitro models and drug treatment responses. A) HUVEC/NHDF co-culture model with Sunitinib (100 nM) and VEGF (100 ng/mL) treatment. B) ECFC/ADSC model with Sunitinib (100 nM) and VEGF (100 ng/mL) treatment. (Scale bars, 200µm).

Figure 10: Summarized model performance and drug treatment responses. A) HUVEC/NHDF co-culture model with Sunitinib (100 nM) and VEGF (100 ng/mL) treatment. B) ECFC/ADSC model with Sunitinib (100 nM) and VEGF (100 ng/mL) treatment. (Scale bars, 200µm).