Cellular Dynamics international

Application Note

iCell® Endothelial Cells:

Assaying Cell Proliferation

iCell® Endothelial Cells, derived from human induced pluripotent stem cells, represent an optimal in vitro test system for vascular biology interrogations in academic research as well as in drug development.

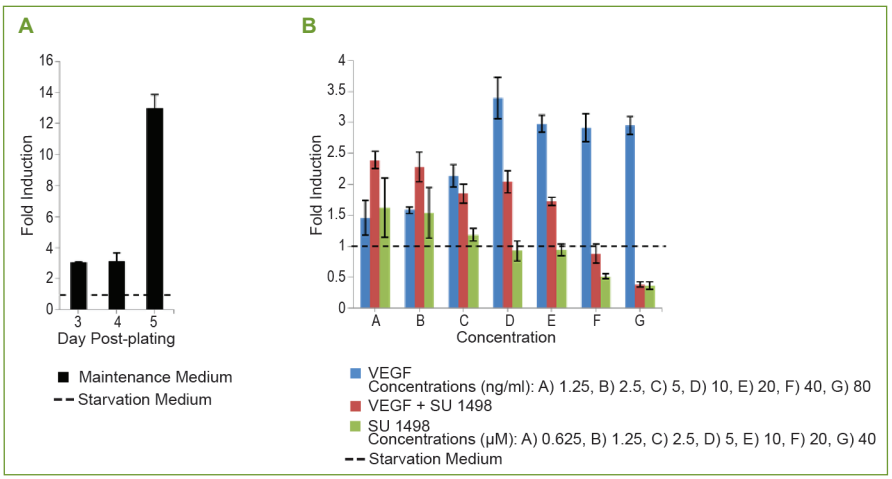
Regulation of endothelial cell proliferation plays a fundamental role in vascular remodeling and angiogenesis in several physiological and pathological conditions. To demonstrate the use of endothelial cells in proliferation assays, iCell Endothelial Cells were treated with a growth factor and/or its receptor inhibitor to modulate a known pro-angiogenic signaling pathway. The proliferation of iCell Endothelial Cells was assessed using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega), a homogeneous method based on the quantitation of ATP in metabolically active cells (1).

Methods

A 96-well cell culture plate (Corning, #3603) was pre-coated with fibronectin (3 µg/cm^2, Invitrogen #33016-015) at room temperature for 1 hour. iCell Endothelial Cells (>95% purity) were seeded in Complete iCell Endothelial Cells Maintenance Medium (Maintenance Medium) at 5,000 cells/cm^2 to provide ~1,600 cells/well in a final volume of 100 µl/well. The plate was transferred to an incubator at 37˚C, 5% CO_2, 5% O_2. 24 hours post-plating, the medium was exchanged with 100 µl/well of fresh Maintenance Medium or Starvation Medium (VascuLife Basal Medium with 4 mM L-glutamine LifeFactor and 0.1% iCell Endothelial Cells Medium Supplement). Cells grown in Maintenance Medium were assayed at 72, 96, and 120 hours (days 3, 4, and 5) post-plating (Figure 1A), with an additional fresh medium exchange at 96 hours post-plating. 48 hours post-plating, cells in Starvation Medium were fed with 100 µl/well of fresh Starvation Medium with a seven-point titration (2-fold dilution series) of VEGF (highest concentration of 80 ng/ml), VEGFR2-inhibitor (Tyrphostin SU 1498, Sigma, #4192, highest concentration of 40 µM), or VEGFR2-inhibitor (Tyrphostin SU 1498, highest concentration of 40 µM) in the presence of 5 ng/ml of VEGF. 48 hours after treatment, the cells were fed with fresh Starvation Medium containing the same compound titrations and assayed the following day (day 5 post-plating, Figure 1B). The amount of cellular ATP was quantified using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, #G7571) per the manufacturer's instructions. Luminescence readings were taken using the Tecan GENios Pro Microplate Reader (1 second integration time/sample). Proliferation fold induction was calculated relative to the values obtained with cells maintained in Starvation Medium (dotted line). Standard deviation was calculated from triplicate wells.

Results & Discussion

VEGF is a known regulator of endothelial cell proliferation and migration acting through two receptors, VEGFR1 and VEGFR2, the latter being suggested as the primary transducer of VEGF during physiological angiogenesis (2). During the proliferation assay, iCell Endothelial Cells plated at low density showed a rapid expansion after 5 days of culture in Maintenance Medium (Figure 1A). In serum starvation conditions, proliferation induction of iCell Endothelial Cells by 3-day treatment with VEGF was dose-dependent and reached a maximum effect at 10 ng/ml (concentration D), followed by a plateau at higher concentrations (Figure 1B). The presence of VEGFR2-inhibitor, Tyrphostin SU 1498, in combination with 5 ng/ml of VEGF, resulted in a decreased proliferation induction of iCell Endothelial Cells at 20 and 40 µM (concentrations F and G).



▲ **Figure 1: Cell Proliferation Assay Using iCell Endothelial Cells**
A) iCell Endothelial Cells were cultured for 5 days in iCell Endothelial Cells Maintenance Medium (Maintenance Medium) and assayed at days 3, 4, and 5 post-plating, showing a rapid expansion after 5 days of culture. B) iCell Endothelial Cells were cultured for 1 day in Maintenance Medium, serum-starved for 1 day, treated with a seven-point titration of VEGF and/or its receptor inhibitor (SU 1498) for 3 days, and assayed at day 5 post-plating.

Conclusion

iCell Endothelial Cells proliferate in response to VEGF stimulation through VEGFR2 signaling, which can be modulated by the addition of a selective receptor inhibitor. Growth factor-induced proliferation activity can be reliably assessed on iCell Endothelial Cells using the CellTiter-Glo Luminescent Cell Viability Assay.

References

1. Promega Technical Bulletin MultiTox-Fluor Multiplex Cytotoxicity Assay #TB348.
2. Waltenberger J, Claesson-Welsh L, Siegbahn A, et al. Different Signal Transduction Properties of KDR and Flt1, Two Receptors for Vascular Endothelial Growth Factor. J Biol Chem 269, 26988-95 (1994).

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