xCELLigence System Real-Time Cell Analyzer

Focus Application
Cell Migration

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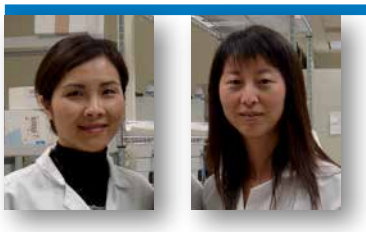
Featured Study:
Automated Continuous Monitoring of Growth Factor-Mediated Endothelial Cell Migration using the CIM-Plate 16 and xCELLigence RTCA DP Instrument



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Introduction

Cell migration and invasion are mechanically integrated molecular processes and fundamental components of embryogenesis, vasculogenesis, immune responses, as well as pathophysiological events such as cancer cell metastasis (1, 2). Cell migration and invasion involve morphological changes due to actin cytoskeleton rearrangement and the emergence of protrusive membrane structures followed by contraction of the cell body, uropod detachment, and secretion of matrix degrading enzymes (1, 2). These multi-step processes are influenced by extracellular and intracellular factors and signaling events through specialized membrane receptors.

The integrated nature of cell migration is exemplified by angiogenesis. Angiogenesis or neo-angiogenesis refers to the formation of new blood vessels from pre-existing vessels and is critical for development, wound healing and tumor growth. Endothelial cell migration is an important component of angiogenesis, involving chemotactic, haptotactic and mechanotactic (shear stress) induced cell migration (3). Chemotactic cell migration is typically induced by soluble growth factors such as vascular endothelial growth factor (VEGF) and its isoforms, fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF) amongst others. These growth factors interact with their cognate receptor tyrosine kinases on he surface of endothelial cells activating signaling pathways culminating in directed cell migration.

In the present study, we used the new CIM-Plate 16 with the xCELLigence RTCA DP Instrument to monitor growth factor-mediated migration of endothelial cells in realtime using label-free conditions. The CIM-Plate 16 is a 16-well modified Boyden chamber composed of an upper chamber (UC) and a lower chamber (LC). The UC and LC easily snap together to form a tight seal. The UC is sealed at its bottom by a microporous Polyethylene terephthalate (PET) membrane. These micropores permit the physical translocation of cells from the upper part of the UC to the bottom side of the membrane. The bottom side of the membrane (the side facing the LC) contains interdigitated gold microelectrode sensors which will come in contact with migrated cells and generate an impedance signal. The LC contains 16 wells, each of which serves as a reservoir for a chemoattractant solution on the bottom side of the wells, separated from each other by pressure-sensitive O-ring seals.

Results

To analyze endothelial cell migration using the CIM-Plate 16, human umbilical vein endothelial cells (HUVEC) from Lifeline Cell Technologies were cultured in Vasculife VEGF cell culture medium, according to the manufacturer's recommendations. Cells were serum starved in Vasculife Basal medium, detached using a trypsin-EDTA solution, and the cell density was adjusted to 300,000 cells/mL.
To assess general HUVEC cell migration in response to

different growth factors encountered during angiogenesis, Vasculife VEGF medium containing VEGF, EGF, IGF, or bFGF with 2% fetal bovine serum, was serially diluted with Vasculife Basal medium and transferred to the lower chamber of the CIM-Plate 16 (see Figure 1). For optimal HUVEC migration, it was determined from previous experiments that extracellular matrix (ECM) proteins, such as fibronectin (FN) are necessary. The PET membrane was therefore coated on both sides with 20 µg/mL FN. After CIM-Plate 16 assembly, 100 µL of cell suspension (30,000 cells) were added to each well of the UC. The CIM-Plate 16 was placed in the RTCA DP Instrument equilibrated in a CO_2 incubator. HUVEC migration was continuously monitored using the RTCA DP Instrument. Figure 1 shows the time- and dose-dependent directional migration of HUVEC cells from the upper chamber to the lower chamber. The combination of growth factors and serum provides a strong chemoattractant signal which together induce the directional migration of HUVEC cells through the micropores of the CIM-Plate 16. Migrating cells are detected by the electronic sensing microelectrodes, producing changes in the measured Cell Index values (see Figure 1). HUVEC migration has been shown to be influenced by a number of growth factors including VEGF, HGF, and bFGF. These growth factors are known to be secreted by tumors and cells within the tumor stroma, as well as endothelial cells inducing migration and angiogenesis.

To measure HUVEC migration in response to individual growth factors using the CIM-Plate 16, HUVEC cells from Lonza were starved for 6 hours and detached. At the same time, titration of HGF, and separately of VEGF, was performed in basal media (complete HUVEC media diluted at a ratio of 1:125 with EGM media from Lonza). Each of these growth factors was then transferred to the wells of the lower chamber (see Figure 2). The PET membrane was coated with FN as described above.

After CIM-Plate 16 assembly, HUVEC cells were added at 30,000 cells/well for VEGF-induced migration and 15,000 cells/well for HGF-induced migration. Time-dependent HUVEC migration was monitored using the RTCA DP Instrument. Both VEGF and HGF induced the migration of HUVEC cells in a time- and dose-dependent manner (see Figure 2A and 2B). The RTCA Software 1.2 was used to calculate time-dependent EC_{50} values for both VEGF- and HGF-mediated HUVEC migration (see Figure 2C and 2D).

Angiogenesis is a compelling target for cancer therapy. Monoclonal antibodies targeting angiogenesis play an important role in colon and lung cancer therapy (4). For this reason, the migration of endothelial cells in response to angiogenic factors such as VEGF is a good *in vitro* model system for studying and screening potential inhibitors of this process (see Figure 3). For the quantitative and time-dependent assessment of inhibition of VEGF-induced endothelial cell migration, HUVEC cells were added to the CIM-Plate 16, as described above, in the presence of increasing amounts of a VEGF receptor inhibitor. As shown in Figure 3A, this inhibitor was found to potently block VEGF-induced cell migration in a time- and dose-dependent manner. The inhibition of VEGF-induced HUVEC cell migration by this compound was quantified using the RTCA Software 1.2. Time-dependent IC_{50} values, shown in Figure 3B, demonstrate that this particular VEGF receptor inhibitor blocks the kinase activity of all three VEGF receptor isoforms with IC_{50} values in the picomolar range.

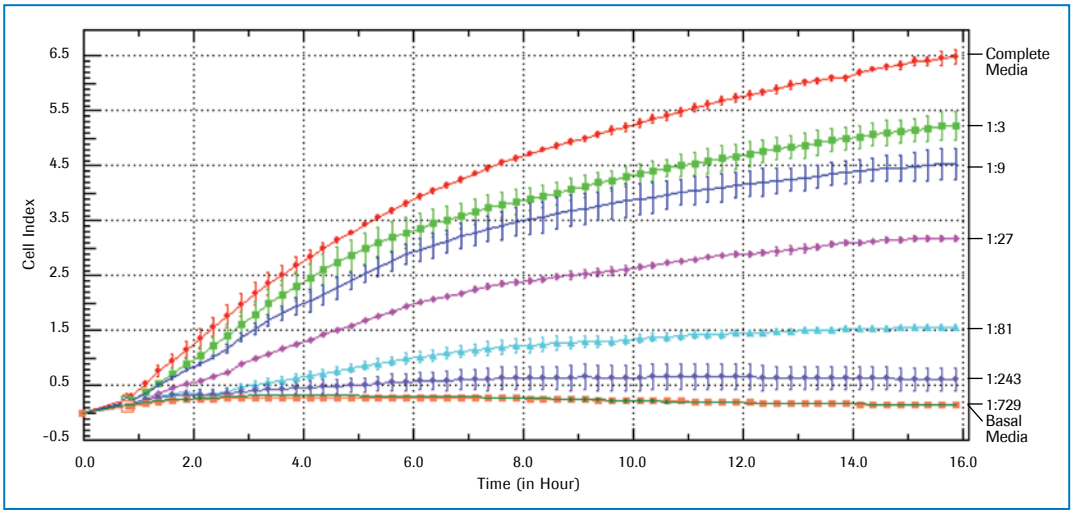

Figure 1: Time- and dose-dependent directional migration of HUVEC cells from the upper to the lower CIM-Plate 16 chamber. To assess HUVEC cell migration, Vasculife VEGF medium, containing VEGF, EGF, IGF, bFGF and 2% fetal bovine serum, was serially diluted with Vasculife Basal medium and transferred to the lower chamber of the CIM-Plate 16.

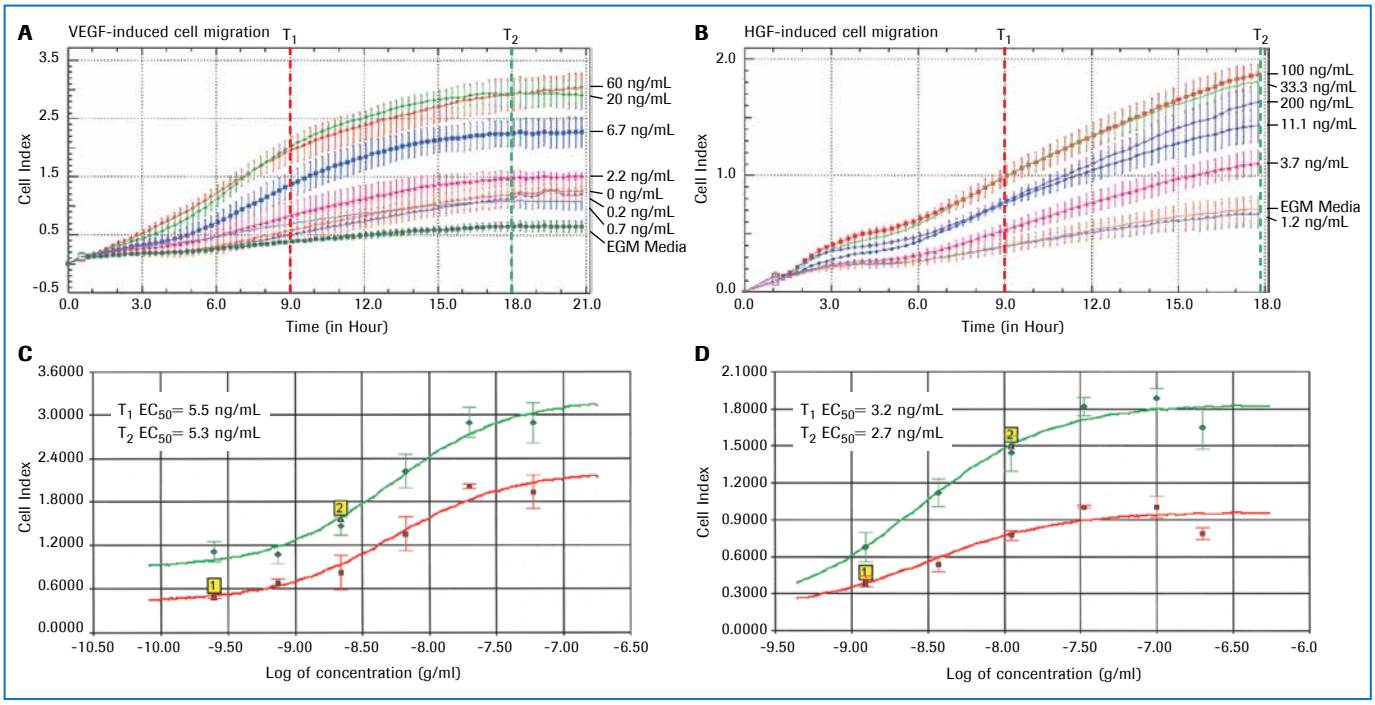

Figure 2: HUVEC cell migration in response to the growth factors, VEGF (A, C) and HGF (B, D) using the CIM-Plate 16 and xCELLigence RTCA DP Instrument, showing Cell Index profiles (A, B) and EC_{50} plots (C, D); see text in Results for details.

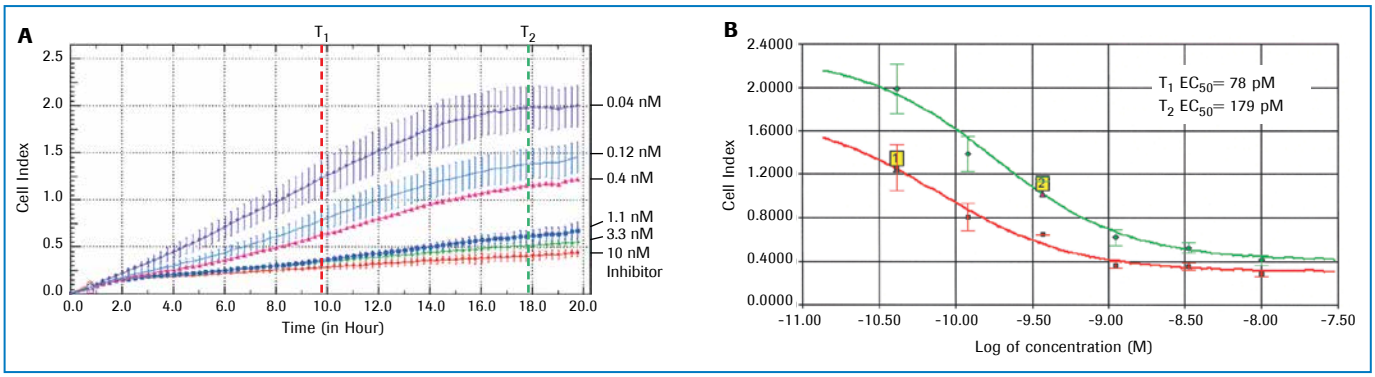

Figure 3: Real-time continuous HUVEC cell monitoring showing the Cell Index profiles (A) and IC_{50} plot (B) for the inhibition of VEGF-induced cell migration by a VEGF receptor inhibitor; see text in Results for details.

Conclusion

Data presented in this application note demonstrate that growth-factor-mediated endothelial cell migration can be monitored quantitatively and in realtime using the CIM-Plate 16 with the RTCA DP Instrument. The xCELLigence System proved to be ideal for assessing and screening an inhibitor of endothelial cell migration and angiogenesis. The CIM-Plate 16 combines the benefits of continuous label-free impedance-based technology with the classic Boyden chamber permitting automated, real-time, and quantitative measurements of cell migration and invasion.

Classic cell migration techniques utilizing standard and transwell Boyden chambers are labor intensive, producing results that can be difficult to reproduce. The non-invasive CIM-Plate 16 does not require manual cell counting or cell labeling. Moreover, the continuous real-time data obtained using the CIM-Plate 16 identifies optimal time points for performing parallel gene expression and functional analyses of HUVEC migration. The above described features and benefits of the CIM-Plate 16 and the RTCA DP Instrument describe an ideal system for the *in vitro* analysis of the cellular and molecular events associated with cell migration and invasion.

References

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Ordering Information

Product	Cat. No.	Pack Size
xCELLigence RTCA DP Instrument	**00380601050**	**1 Bundled Package**
RTCA DP Analyzer	05469759001	1 Instrument
RTCA Control Unit	05454417001	1 Notebook PC
E-Plate 16	05469830001	6 Plates
	05469813001	6 x 6 Plates
E-Plate VIEW 16	06324738001	6 Plates
	06324746001	6 x 6 Plates
E-Plate Insert 16	06465382001	1 x 6 Devices (6 16-Well Inserts)
CIM-Plate 16	05665817001	6 Plates
	05665825001	6 x 6 Plates

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Key Words:
Growth factor-mediated cell migration, xCELLigence System, RTCA DP Instrument, real-time migration monitoring, CIM-Plate 16, non-invasive and label-free migration detection

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