xCELLigence® System Applications
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Different Cell Lines Produce Different Time-Dependent Impedance Profiles

Cells were seeded on an E-plate 96 at the indicated cell density and monitored for overall impedance profile over several days. (Data and figures adapted from ACEA Biosciences, unpublished data).

Quantitative Assessment of Profile Differences Can Identify Cell Quality Issues

(A) Morphology of HeLa isolates. HeLa cell lines obtained from five different laboratories and from ATCC were fixed, stained, and imaged by bright-field microscopy. (B) 5000 cells were seeded on an E-plate 96 and monitored continuously for 50 hours. (C) and (D) Distinct groupings based on the quantitative assessment of specific parameters reflected cell line mix-ups, genetic changes, and non-genetic changes attributed to passage number effects.

(*) = Statistically significant difference from ATCC control (P<0.05).
(**) = Statistically indistinguishable group (P>0.05).
(Data and figures adapted from Irelan JT, et. al., 2011).
SELECT PUBLICATIONS FOR CELL QUALITY CONTROL


2. ATCC technology assessment of Roche xCELLigence System: an electronic impedance-based cell sensing unit.
   Langenbach K.

3. Live cell quality control and utility of real-time cell electronic sensing for assay development.

KEY BENEFITS

- Different cells produce different time-dependent impedance profiles.
- These profiles offer a distinct “fingerprint” reflecting overall cell behavior.
- These profile differences reflect different adhesion, morphology, and growth rates.
- Cell quality issues, including cell line mix-ups, genetic changes, contamination, and passage number effects, result in distinct impedance profile changes.
- Each of these parameters may be rapidly and quantitatively assessed using the xCELLigence System.
Kinetic Response Profiles Reflect Different Cytotoxic Mechanisms

HepG2 cells were treated with various cytotoxic compounds and monitored in real time for cytotoxic response kinetics. Representative compounds targeting tubulin (paclitaxel), actin (cytochalasin D), DNA (doxorubicin), mitochondria (rotenone), calcium homeostasis (thapsigargin), and protein transport (brefeldin A) generated distinct response profiles. (Data and figure adapted from ACEA Biosciences, Inc. unpublished data).

Real Time Monitoring Allows Identification of Optimal Timepoints for Further Analysis

HeLa cells were treated with cytotoxic compounds and monitored in real time for cytotoxic response kinetics on xCELLigence. At the indicated timepoints, cells in a parallel plate were analyzed using the WST-1 cell viability assay. 5-FU or MG132 caused different dose-dependant kinetic response profiles with different optimal times for performing the WST-1 assay. (Data and figure adapted from Ke N, et. al., 2011).
SELECT PUBLICATIONS FOR DRUG-MEDIATED CYTOTOXICITY/APOPTOSIS

1. **The xCELLigence system for real-time and label-free monitoring of cell viability.**
   Ke N, Wang X, Xu X, Abassi Y.

2. **Kinetic cell-based morphological screening: prediction of mechanism of compound action and off-target effects.**

3. **Dynamic monitoring of cytotoxicity on microelectronic sensors.**
   Xing JZ, Zhu L, Jackson JA, Gabos S, Sun XJ, Wang XB, Xu X.

KEY BENEFITS

- Kinetic responses can be predictive of mechanism of action.
- Continuous monitoring ensures no meaningful time points are missed.
- Real-time data allows identification of the optimal times for treatment and data collection.
- Non-invasive assay is performed in tissue culture incubator, allowing for analysis by standard viability assays at any point during the experiment.
- Easy quantification of the onset and kinetics of the cytotoxic response.
T Cells’ Non-Adherent Property is Useful in Cytolytic Assays

(A) A schematic diagram of Cell-Mediated Cytolysis on E-Plate wells. (B) Tumor cells (red) not T cells (blue) elicit impedance. (C) Impedance measurements are transiently disrupted with the addition of T cells to tumor cells. The trace for tumor cells alone is shown in red. The other traces represent tumor cells co-cultured with HER-2/neu p369-specific T cells (Blue: ratio of 1.25 T cells/tumor cell, Green: ratio of 40 T cells/tumor cell). Results are representative of 3 separate experiments. (Data and figures adapted from Erskine CL, et. al., 2012).

Killer T Cell Activity is Dose Dependent

(A) Reduction in impedance mediated by T cells is dose dependent. The impedance traces of SKBR3 tumor cells incubated alone or with varying concentrations of tumor-specific T cells. Each trace is calculated from triplicate data points collected every 5 minutes through the duration of the culture. Results are representative of 3 separate experiments. (B) The impedance based assay is more sensitive than the chromium release assay for the detection of tumor antigen-specific T cells. Shown are % lysis of SKBR3 in response to varying concentrations of p369-specific T cells as measured at 5 hours following tumor and T cell mixing using both the impedance-based assay and the chromium release assay. (Data and figures adapted from Erskine CL, et. al., 2012).
Comparison of Cell Index and $[^3]$H]thymidine Incorporation in NK-92-mediated Killing

(A) Representative changes of CI after plating of MCF-7 cells. After 13 h the MCF-7 cells [8,000 alone (black) or plus NK-92 cells at an effector:target cell ratio of 2:1 (red), 5:1 (green), or 20:1 (blue)] were pulsed-labeled with 0.5 µCi of $[^3]$H]thymidine (left arrow) for 5 h before NK-92 cells were added. (B) At different times after NK-92 addition, the E-plates were washed with medium, CI was measured, and subsequently the amount of radioactivity was determined. The $[^3]$H]thymidine content of MCF-7 cells is shown at different time points [2.5 h (black column), 4.5 h (dark gray column), 6.0 h (light gray column), and 23 h (white column)] at three NK-92 densities (effector:target 0:1, 5:1, and 20:1) (n = 4). Statistical analysis (analysis of variance) showed that the decrease of $[^3]$H]thymidine content was highly significant (P < 0.001) for all time points except 2.5 h. CPM, counts per minute. (Data and figures adapted from Glamann J, et. al., 2006).

Detection of Antibody Dependent Cell-mediated Cytolysis (ADCC)

(A) The changes of CI (normalized) before and after inducing ADCC by adding Erbitux and bulk clone NK cells to 16 e-plates devices seeded with A431 cells (8,000 cells per well) at time 0. The left arrow marks addition of antibodies, and the right arrow indicates the addition of NK cells (160,000 per well; 20:1 effector:target cell ratio). (B) The concentration–response curve of Erbitux (50% effective concentration 1.3 ng/ml) 10 h after addition of NK cells. (Data and figures adapted from Glamann J, et. al., 2006).
CELL-MEDIATED CYTOTOXICITY AND ADCC

Comparison of LAK Cell Killing and Development of NK Cytotoxicity

(A) Lymphokine activated NK (LAK) cell killing of NT2A2 by four different NK donors. The addition of the IL-2 activated NK cells is marked by the vertical bar at 23.30 h. It shows the profile of killing across the 80 h time course. (For the generation of LAK-activity, NK cells were cultured in the presence of human recombinant IL-2 (Peprotech) 50 ng/ml for 20 h.) (B) Development of NK cytotoxicity. The influence of the target NT2As on the generation LAK-activity was investigated by adding IL-2 (50 ng/ml) to NK-astrocyte co-cultures. (The IL-2 was added to the co-cultures 5 min after the NK cells were seeded onto the target NT2As.) (Data and figures adapted from Moodley K, et al., 2011).

Measuring Potency of Bispecific Antibody (XGFR) Targeting IGF-1R and EGFR

(A) The schematic diagrams show the one-arm single chain Fab bispecific antibody XGFR, and the XGFR2 antibody with C-terminal attachment of disulfide-stabilized scFvs, and the dual V domain (DVD) antibody XGFR-DVD. All VH and VL domains in the bispecific antibodies are derived from the parental antibodies GA201 (EGFR; red and yellow) and R1507 (IGF-1R; blue and light blue). (B) Cells were incubated at an effector/tumor cell ratio of 3:1 for 5 h at the indicated concentrations of XGFR, XGFR2, and the parental control antibodies R7072 and GA201 in triplicate in two or more independent experiments. The xCELLigence technology and software was used for data analysis. (Data and figures adapted from Schanzer JM, et. al., 2014).
SELECT PUBLICATIONS FOR CELL-MEDIATED CYTOTOXICITY AND ADCC

1. **Novel bispecific antibodies increase γδ T-cell cytotoxicity against pancreatic cancer cells.**

2. **A Novel Glycoengineered Bispecific Antibody Format for Targeted Inhibition of Epidermal Growth Factor Receptor (EGFR) and Insulin-like Growth Factor Receptor Type I (IGF-1R) Demonstrating Unique Molecular Properties.**

3. **Enzymatic discovery of a HER-2/neu epitope that generates cross-reactive T cells.**
   Henle AM1, Erskine CL, Benson LM, Clynes R, Knutson KL.

4. **Epigenetic modulation to enable antigen-specific T-cell therapy of colorectal cancer.**
   Chou J, Voong LN, Mortales CL, Towlerton AM, Poliack SM, Chen X, Yee C, Robbins PF, Warren EH.

5. **Determining optimal cytotoxic activity of human Her2neu specific CD8 T cells by comparing the Cr51 release assay to the xCELLigence system.**
   Erskine CL, Henle AM, Knutson KL.

6. **Real-time profiling of NK cell killing of human astrocytes using xCELLigence technology.**
   Moodley K, Angel CE, Glass M, Graham E.

7. **Pertuzumab in combination with trastuzumab shows significantly enhanced antitumor activity in HER2-positive human gastric cancer xenograft models.**

8. **Unique functional status of natural killer cells in metastatic stage IV melanoma patients and its modulation by chemotherapy.**
   *Cancer Res.* 2011;17(9):2628-37.

9. **Dynamic detection of natural killer cell-mediated cytotoxicity and cell adhesion by electrical impedance measurements.**
   Glamann J and Hansen AJ.,

**KEY BENEFITS**

- Real-time monitoring of cell-mediated cytotoxicity, allows measurement of both fast killing kinetics (hrs) and slow killing kinetics (days). Whereas the standard endpoint assays such as the LDH assay and Cr51 release assay can only focus on short term (hrs) cytolysis.
- Label-free, requires less cells compared to the conventional Cr51 release assay, which needs to label target cells with radioactive isotopes.
- Direct, sensitive and specific measurement of target cell changes. This is in contrast to the assays based on release of nonradioactive compounds from effector cells.
- Homogeneous assay. This is in comparison to the ATP production assays that require to wash effector cells out of wells first.
- Non-labor intensive assay compared to laborious FACS analysis.
Quantitative Assessment of Extracellular Matrix Effect on A549 Attachment and Spreading

The Cell Index increases proportionately as the coating concentration of collagen IV increases. (Data and figures adapted from ACEA Biosciences, unpublished data).

Mammary Stem-Like Cells Have Increased Tubulin-Dependent Initial Reattachment From Suspension

(A) Stem-like HMLE cells attach at significantly faster rates than non-stem-like HMLE cells as determined by electrical impedance expressed as Cell Index. Stem-like HMLE and non-stem-like cells both have significantly reduced attachment when treated with the microtubule polymerization inhibitor colchicine (50µM). For all reattachment assay: lines, mean for quadruplicate wells; bars, SD; representative graph from three independent experiments is shown. (B) Phase contrast images of HMLE non-stem-like (a-d) and stem-like (e-h) subpopulations reattaching from suspension. Panels, 10x magnification; Insets, 60x magnification. (Data and figures adapted from Charpentier MS, et al., 2014).
SELECT PUBLICATIONS FOR CELL ADHESION AND SPREADING

1. Curcumin targets breast cancer stem-like cells with microtentacles that persist in mammospheres and promote reattachment.
Charpentier MS, Whipple RA, Vitolo MI, Boggs AE, Slovic J, Thompson KN, Bhandary L, Martin SS.


3. A role for adhesion and degranulation-promoting adapter protein in collagen-induced platelet activation mediated via integrin a2b1.
Jarvis GE, Bihan D, Hamaia S, Pugh N, Ghevaert CJ, Pearce AC, Hughes CE, Watson SP, Ware J, Rudd CE, Farndale RW.

4. Dynamic monitoring of cell adhesion and spreading on microelectronic sensor arrays.
Josephine M. Atienza, Jenny Zhu, Xiaobo Wang, Xiao Xu and Yama Abassi.

KEY BENEFITS

- Real-time monitoring of cell adhesion and spreading.
- Label-free assay requires no fixation, staining or any other sample processing.
- Direct, sensitive, and quantitative.
- Easy quantification of the kinetics of adhesion and spreading.
- Rapid optimization of cell density and extracellular matrix coating conditions.
Monitoring Functional Activation of $G_s$ and $G_i$ Subtypes

Pre-treatment of $\beta_2$AR with selective $G_s$ and $G_i$ inhibitors cholera toxin (CTX) and pertussis toxin (PTX), respectively, modulates the kinetic impedance profile of $\beta_2$AR selective agonist isoproterenol (ISO). Impedance encapsulates a holistic readout that captures the contribution of multiple G protein dependent signaling events to a cellular response. The rapid drop in cell index is delayed and reduced by PTX and to a lesser extent CTX, while both treatments largely block the longer term increase in cell index, indicating differential involvement of $G_s$ and $G_i$ signaling. (Data and figures adapted from Stallert W, et. al., 2012)

Assessment of $G_{\beta\gamma}$-dependent Signaling

Inhibition of $G_{\beta\gamma}$-dependent signaling with gallein (Gall) demonstrates a reduced kinetic ISO-promoted impedance response. A slower ascending phase and partially decreased maximum response suggest a distinct role for $G_{\beta\gamma}$-dependent signaling. (Data and figures adapted from Stallert W, et. al., 2012)
SELECT PUBLICATIONS FOR GPCR-MEDIATED CELL SIGNALING


KEY BENEFITS

- Simultaneous screening for GPCR function across all coupling classes: G_s, G_i, G_q, G_{12/13}.
- Detection of traditionally difficult classes: G_i and G_{12/13}.
- Detection of functional selectivity.
- Potential advantage to de-orphaning of GPCRs.
- Assay endogenous GPCRs with primary cells, stem cells, and/or other disease relevant cells.
Endogenous Receptor Tyrosine Kinase Short Term Response

(A) Cells expressing recombinant PDGFRb were seeded on an E-plate 96 at 20,000 cells per well and after overnight growth the medium was replaced with serum free medium (not shown). (A) After 2 hours cells were treated with increasing doses of the PDGF inhibitor Imatinib, incubated for one hour, then stimulated with 10 ng/ml PDGF BB. (B) Imatinib showed a dose-responsive inhibition of the morphology change resulting from PDGF stimulation, with an IC50 value of 135 nM as determined using the xCELLigence software (Data and figures adapted from ACEA Biosciences, unpublished data).

Endogenous Receptor Tyrosine Kinase Expressing Cell Line Response to Inhibitor

(A) Cells expressing constitutively active cMET were seeded on an E-plate 384 at 10,000 cells per well and after overnight growth cells were treated with increasing doses of the cMET inhibitor Crizotinib. (B) Crizotinib showed a dose-responsive morphology change resulting from blocking the constitutive cMET signal, with an IC50 value of 87.5 nM as determined using the xCELLigence software (Data and figures adapted from ACEA Biosciences, unpublished data).
SELECT PUBLICATIONS FOR RTK-MEDIATED CELL SIGNALING


2. Modeling ERBB receptor-regulated G1/S transition to find novel targets for de novo trastuzumab resistance.

3. Label-free and real-time cell-based kinase assay for screening selective and potent receptor tyrosine kinase inhibitors using microelectronic sensor array.

KEY BENEFITS

- The integrated, global response of cells to RTK modulation can be monitored.
- Rapid responses involving cell morphology and attachment changes may be assayed on a minute timescale.
- Slower responses involving cell proliferation and viability may be assayed over days or weeks.
- Receptor activity can often be assessed in the endogenous context, in a disease-relevant cell type.
RECEPTOR-MEDIATED SIGNALING: NHR

Estrogen Specific Response Monitored on xCELLigence System

The estrogen receptor agonist 17β-estradiol (E2) induced a unique kinetic response profile with delayed cell index growth in the human breast cancer cell line T-47D. Such response was abolished by the estrogen receptor antagonist ICI182780 (A). The progesterone antagonist mifepristone cannot abolish E2 response (B), but rather had a synergistic effect with E2 on T-47D cells. (Data and figures adapted from Jin C, et. al., 2011).

Progesterone Specific Response Monitored on xCELLigence System

Progesterone induced unique kinetic response profile with a biphasic cell index curve in the human breast cancer cell line T-47D. This response was blocked by the progesterone antagonist mifepristone (A), but could not be abolished by the estrogen receptor antagonist ICI182780 (B). (Data and figures adapted from Jin C, et. al., 2011).
SELECT PUBLICATIONS FOR NUCLEAR HORMONE RECEPTOR

1. **Real-Time Growth Kinetics Measuring Hormone Mimicry for ToxCast Chemicals in T-47D Human Ductal Carcinoma Cell.**

2. **The Aryl Hydrocarbon Receptor Mediates Leflunomide-Induced Growth Inhibition of Melanoma Cells.**

3. **A Novel Sensitive and Selective Real-time Cellular Assay for Detection of Endocrine Disruptors Using Native Endocrine Signaling Pathways.**
   Jin, C., Abassi, Y., Xu, X. & Wang X.

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**KEY BENEFITS**

- Label-free nature allows for sensitive detection of endogenous receptor activity.
- Kinetic response profiles may be diagnostic for specific pathways.
- Ability to differentiate cytotoxicity and proliferation with a single experiment.
- Real time data can identify the optimal time to monitor different ligand effects with standard assay.
Cell-Response Profiling: Small Molecule and siRNA

Using the xCELLigence System for Target Identification and Validation

(A) HeLa cells were transfected with various concentrations of KIF11 (the gene encoding Eg5) siRNA. A time and concentration-dependent antimitotic time-dependent cell response profiles (TCRPs) was observed with higher siRNA concentrations producing more pronounced CI changes. The kinetics of the CI profiles is very similar between various concentrations of siRNAs: CI for the transfected samples started to diverge from the control samples starting 9-12 h post transfection, reaching the lowest level approximately 24 h post transfection, before starting to recover, indicating the specificity of the TCRP. KIF11 gene expression is supported with analysis of Eg5 protein expression by Western blotting (B). (Data and figures adapted from Ke N, et. al., 2010).

Using “Signature” TCRPs to Identify Compound Properties for Screening

Compounds with similar mechanisms of action often have similar TCRPs. (A) In A549 cells, both anti-mitotic compounds, paclitaxel and vincristine, mediated the signature anti-mitotic response profile; the DNA damaging agent 5-FU mediated a cytostatic pattern, while the topoisomerase inhibitor camptothecin mediated pronounced cytotoxicity. (B) Cell Index values are consistent with bright-field imaging in the E-Plate View area. (Data and figures adapted from iCELLigence System Application Note No.2, 2012).
1. **Identification of genomic alterations in oesophageal squamous cell cancer.**
*Nature.* 2014 May 1;509(7498):91-5.

2. **Time-resolved human kinome RNAi screen identifies a network regulating mitotic-events as early regulators of cell proliferation.**

3. **Screening and identification of small molecule compounds perturbing mitosis using time-dependent cellular response profiles.**

4. **RNAi phenotype profiling of kinases identifies potential therapeutic targets in Ewing’s sarcoma.**

3. **BH3 response profiles from neuroblastoma mitochondria predict activity of small molecule Bcl-2 family antagonists.**
*Cell Death Differ.* 2010 May;17(5):872-82.

6. **Kinetic cell-based morphological screening: prediction of mechanism of compound action and off-target effects.**

**KEY BENEFITS**

- Real-time monitoring of kinetics of cell responses to treatment can reveal novel information regarding mechanism of action.
- Assays are performed in tissue culture incubator, allowing for detection of long-term effects.
- Label-free assay requires no fixation, staining or any other sample processing.
- Cell response profiles can allow for early identification of unexpected off-target or toxic effects of treatments.
Real-time Monitoring of West Nile Virus (WNV) Induced CPE on Vero Cells

(A) Normalized cell index plotted as a function of time in hours post infection for wells inoculated with different plaque forming units (PFU) of virus and control (ctrl) wells without virus addition. The horizontal line depicts the 50% point in the decline of the Cell Index(CI), defined here as the CIT50 value. (B) CIT50 values were regressed as a function infectious dose in log10 PFU. (Data and figures adapted from Fang Y., et. al., 2011).

Quantitative Detection of WNV Neutralizing Antibody Titer Using Real-Time Monitoring of Virus-Induced CPE

(A) Virus-induced CPE kinetic pattern of Vero cells presented with 106 PFU of WNV incubated with different titers of neutralizing antibody; control (ctrl), no infection. The horizontal line indicates the 50% decline of CI value. (B) Linear regression between CIT50 value and the neutralizing antibody titer. (Data and figures adapted from Fang Y., et. al., 2011).
SELECT PUBLICATIONS FOR VIRUS MEDIATED CPE

1. **Real-time cell analysis--a new method for dynamic, quantitative measurement of infectious viruses and antiserum neutralizing activity**
   Teng Z, Kuang X, Wang J, Zhang X...

2. **Novel, real-time cell analysis for measuring viral cytopathogenesis and the efficacy of neutralizing antibodies to the 2009 influenza A (H1N1) virus.**

3. **Real-time monitoring of flavivirus induced cytopathogenesis using cell electric impedance technology.**
   Fang, Y., Ye, P., Wang, X., Xu, X., & Reisen, W.

**KEY BENEFITS**

- A simple alternative method to plaque test for measuring lytic activity of viruses.
- Provide quantitative information about the onset and kinetics of viral mediated cytopathic effects (CPE).
- Rapidly identify the optimal viral titer and assay time point for subsequent screening of inhibitory compounds, neutralizing antibodies and neutralizing serums.
Comparison of Conventional Methods with the Dynamic Impedance-Based Method for Monitoring Ethanol-Induced Epithelial Barrier Dysfunction

(A) The reversible barrier dysfunction induced by 7.5% ethanol was assessed by measuring phenol red permeability (control: untreated Caco-2 cell monolayer; EtOH: Caco-2 exposed in ethanol for 3 h; EtOH removal: Caco-2 exposed in ethanol for 3 h followed by replacing ethanol with fresh medium for another 3 h) and (B) TEER assay. (C) Dynamic impedance-based monitoring of ethanol (7.5%)-induced epithelial barrier dysfunction, which was reversed after ethanol was removed. Black arrow: ethanol was added; gray arrow: ethanol was removed. (Data and figures adapted from Sun M, et. al., 2012).

Co-Culture of Astrocytes Enhanced the Barrier Function of Brain Microvascular Endothelial Cells (BMEC)

(A) Schematic of assembly of the inverted blood brain barrier model. Astrocytes and BMEC were grown on the transwell membrane in the upper chambers and on the gold electrode in lower chambers the CIM device, respectively. (B) Astrocytes (seeding densities were 0, 1000, 4000 and 8000/well) increased the CI of BMEC in a dose-dependent manner. (Data and figures adapted from Sansing HA, et. al., 2012).
SELECT PUBLICATIONS FOR ENDOTHELIAL BARRIER FUNCTION

1. **CCM1–ICAP-1 complex controls β1 integrin–dependent endothelial contractility and fibronectin remodeling.**

2. **Vinculin-dependent Cadherin mechanosensing regulates efficient epithelial barrier formation.**

3. **An inverted blood-brain barrier model that permits interactions between glia and inflammatory stimuli.**

4. **A dynamic real-time method for monitoring epithelial barrier function in vitro.**

KEY BENEFITS

- A label-free alternative to solute permeability and transendothelial electrical resistance (TEER) assays.
- Direct, sensitive, and quantitative.
- Real-time assay is conducted under normal tissue culture conditions, allowing for monitoring of barrier function disruption as well as recovery.
- Noninvasive nature of the readout allows for orthogonal assays conducted on the same device, including visual monitoring of cell density by microscopy.
The Ability to Capture Data at Millisecond Time Resolution Over Many Hours and Days Allows for Functional Multiplexing

Mouse embryonic stem cell-derived cardiomyocytes (Cor.At cells) were treated with the indicated concentrations of doxorubicin. DMSO (0.1%) serves as vehicle control. (A) General cytotoxicity can be assessed by overall Cell Index measured at long intervals. (B) Changes in the cell beating profile can be assessed by Cell Index measurements taken at millisecond time intervals. Beating rate values are indicated, and changes in amplitude and frequency are readily observed. (Data and figures adapted from Abassi YA, et. al., 2012).

Detection of Arrhythmic Beats Induced by Known Arrhythmogenic Compounds

Human iPS-derived cardiomyocytes (iCells) were treated with the indicated compounds and beating was assessed by Cell Index measurements taken at millisecond time intervals. Known arrhythmogenic compounds were administered as follows: alfuzosin (10μM), cisapride (1μM), dofetilide (0.01μM), erythromycin (30μM), flecainide (3μM), quinidine (10μM), sotalol (100μM), terfenadine (1μM), and thioridazine (3μM). Compounds associated with non-TdP arrhythmia, aconitine (0.03μM) and ouabain (0.03μM), induced tachycardia- or fibrillation-like arrhythmia (0.1%) and 100μM aspirin serve as vehicle and negative control, respectively. (Data and figures adapted from Guo L, et. al., 2011).
SELECT PUBLICATIONS FOR CARDIOSAFETY TESTING

1. Refining the Human iPSC-Cardiomyocyte Arrhythmic Risk Assessment Model.
   Guo, L., Coyle, L., Abrams, R. M. C., Kemper, R., Chiao, E. T., & Kolaja, K. L.

   Himmel, H. M.

3. Cellular impedance assays for predictive preclinical drug screening of kinase inhibitor cardiovascular toxicity.
   Lamore, S. D., Kamendi, H. W., Scott, C. W., Dragan, Y. P., & Peters, M. F.

4. Evaluation of cellular impedance measures of cardiomyocyte cultures for drug screening applications.
   Peters MF, Scott CW., Ochalski R. And Dragan YP.

5. Dynamic monitoring of beating periodicity of stem cell derived cardiomyocytes as a predictive tool for preclinical safety assessment.

6. In vitro model for assessing arrhythmogenic properties of drugs based on high-resolution impedance measurements.
   Nguemo F, Saric T, Pfannkuche K, Watzele M, Reppel M, Hescheler J.

   Guo L., Abrams RMC., Babiarz JE., Cohen JD., Kameoka S., Sanders MJ., Chiao E., Kolaja KL.

8. Impedance-based detection of beating rhythm and proarrhythmic effects of compounds on stem-cell derived cardiomyocytes.
   Jonsson MKB., Wang Q., Becker B.

KEY BENEFITS

- Assess cardiomyocyte contraction in real time under standard tissue culture conditions.
- Validated with mouse and human stem cell derived cardiomyocytes as well as primary rat neonatal cardiomyocytes.
- Cardiomyocyte beating assay reflects functional integration of all components of the excitation – contraction cycle.
- Allows for detection of the effect of certain compounds which may be missed by only electrophysiological techniques.
- Mechanism of toxicity may be predicted by specific waveform profiles, such as arrhythmic beating patterns.
- The system can be used to assess both acute (msec timescale) and long term cardiac liability.
- Avoid the interference of labels (such as calcium binding dyes) with cardiomyocyte function.
Real-Time Monitoring and Image Analysis of Human Skin-Derived Precursor (SKPs) Cell Differentiation

Differentiation of human skin-derived precursors (SKPs) into smooth muscle cells (SMCSs) and undifferentiated SKPs (C) with or without the application of transforming growth factor (TGF)-β1 (A) or TGF-β3 (B) and subsequently fixed and stained to display α-smooth actin, calponin, and SM22α. Real-time analysis correlates the increase in Cell Index with that of morphological differences between the differentiated and undifferentiated SKPs (D). (Data and figures adapted from Steinbach SK, et. al., 2011).

Morphological Changes of Keratinocytes During Terminal Differentiation

(A) Calcium-dependent morphological changes of keratinocytes during terminal differentiation can be exhibited using the RTCA xCELLigence system. (B) The increase in Cell Index correlates with image-based morphological analysis. (Data and figures adapted from Spörl F, et. al., 2010).
1. Optimization and scale-up culture of human endometrial multipotent mesenchymal stromal cells: potential for clinical application.

2. Comparison of long-term retinoic acid-based neural induction methods of bone marrow in human mesenchymal stem cells.


**KEY BENEFITS**

- Real time data reveals the kinetics of cell differentiation.
- Label-free nature minimizes the need for staining with fluorescent marketings involving multiple handling steps for fixation and staining.
- The non-invasive nature of the assay, in combination with the E-Plate VIEW area allows for correlation of real time data with imaging assays.
Real-Time Monitoring of Mast Cell Degranulation in RBL-2H3 Mast Cell Line

(A) Real-time acquisition of impedance-based measurements captures immediate kinetic profile changes of RBL-2H3 when sensitized with IgE and subsequently stimulated with DNP-BSA. (B) Rhodamine-phalloidin staining of sensitized and activated RBL-2H3 cells performed in parallel to the impedance assay indicates that stimulated cells undergo cytoskeleton rearrangements which correlate with the IgE-mediated impedance responses. (Data and figures adapted from Abassi YA., et. al., 2004).

Real-Time Monitoring of T-cell Activation by CD3 and CD28

Dynamic monitoring of T cell activation using the xCELLigence system. (A) Jurkat cells were seeded onto coated E-plates in the presence of indicated concentrations of anti-CD3 and anti-CD28 functional antibodies, shown as cell index (CI). (B) The dose response curve was plotted using the Cell Index Value at the time point indicated with the dotted line in panel A. (C) Effect of mixed anti-CD3 and anti-CD28 antibodies on rearrangement of actin cytoskeleton. Actin staining is shown in green and nuclear staining in blue. The left panel image shows negative control cells and the right panel image shows cells stimulated with anti-CD3 and anti-CD28 functional antibodies at 1 mg/ml. (Data and figure adapted from Guan N, et. al., 2013).
SELECT PUBLICATIONS IMMUNE CELL ACTIVATION

1. Label-free monitoring of T cell activation by the impedance-based xCELLigence system.

2. Label-free, real-time monitoring of IgE-mediated mast cell activation on microelectronic cell sensor arrays.

KEY BENEFITS

- Novel assay platform to support research on allergic inflammation.
- Sensitive and rapid real-time readout for morphology changes associated with immune cell activation.
- Screening for modulators of IgE-function and mast cell activation.
- Eliminate time-consuming, laborious sample processing required by the standard assay.
Real-Time Kinetics of Cellular Response Predicts Compound Mechanism

Clustering analysis of phenotypic screening results on a lung cancer cell line screened against a “known bioactives” compound library. The overall shape of the time-dependent cellular response curve reflects phenotypic responses which may be used to identify mechanism of action of uncharacterised compounds, or novel activities for known bioactives, based on clustering with compounds of known mechanism. (Data and figures adapted from Abassi, YA et al. 2009).

Real-Time Kinetics of Cellular Response Reveals Novel Off-target Effects

A 549 lung carcinoma cells were seeded on an E-plate 96 at 5,000 cells per well and after overnight growth treated with the KIF11 inhibitor Monastrol. An immediate drop in Cell Index indicated an unexpected effect on calcium homeostasis; this was verified using a parallel assay for calcium uptake on HEK293 cells expressing the CAV1.2 voltage-gated calcium channel (red lines). The expected anti-mitotic phenotype resulting from KIF11 inhibition was observed as a long term decrease in Cell Index followed by partial recovery and verified by parallel staining of the mitotic marker Phospho-Histone H3 (blue lines). (Data and figures adapted from Abassi, YA et al. 2009).
SELECT PUBLICATIONS FOR PHENOTYPIC SCREENING

1. **Screening and identification of small molecule compounds perturbing mitosis using time-dependent cellular response profiles.**

2. **Kinetic cell-based morphological screening: prediction of mechanism of compound action and off-target effects.**

KEY BENEFITS

- The integrated, global response of cells to treatment can be monitored.
- The kinetics of cellular response can be predictive of compound mechanism of action.
- The kinetics of cellular response can provide an early alert to possible off-target or toxic effects of a compound.
- Since the assay is non-invasive and conducted under tissue culture conditions, plates may be removed and cells assayed by standard methods at any time for verification of phenotype.
Real-Time Chemotaxis of Bio-Gel Elicited Macrophages to Murine CCL5

(A) Representative trace from the RTCA software of 4×10⁵ Bio-Gel elicited macrophages from C57BL/6J in the top chamber with murine CCL5 (5nM) (red) or no chemokine (green) in the bottom chamber. A control of no macrophages and no chemokine was also run (blue). By 70mins max CI had been reached, monitoring the plate was stopped, removed and membranes were cut and fixed with 2.5% gluteraldehyde and processed for scanning electron microscopy (SEM). Representative images are shown of macrophages adhered to the underside of filters in response to 5 nM murine CCL5 (B,C) no chemokine (D,E) or control where no cells or chemokine were added (F). The number of cells adhered to individual gold discs on the underside of the membrane (seen as light grey regions in the SEM images) were quantitated (n=1 experiment with 5-6 technical replicates of each condition analysed) (G) (Data and figures adapted from Iqbal AJ, et al., 2013).

Assessment of Ovarian Cancer Spheroid Invasion of Mesothelial Cells

(A) Spheroid generation and model of experimental set up. Schematic showing the RTCA 2 chambered CIM plate well set up, in which pre-formed ovarian cancer spheroids are plated on top of a monolayer of an LP9 mesothelial layer/matrix barrier in the upper chamber and media ±FBS as a chemoattractant is added to the lower chamber. Electrodes underneath the interface of the 2 chambers measure increasing electrical impedance as more cells invade through the barriers to the lower chamber. (B - C) Representative results from an RTCA invasion assay conducted with and without FBS in the bottom chamber of a CIM plate well. KGN cell invasion is compared to LP9 mesothelial cells. Results are shown as mean ± SD Cell Index from triplicate wells at the 24 hr timepoint (B) and over an entire two day assay period (C). (Data and figures adapted from Bilandzic M & Stenvers KL, 2014).
SELECT PUBLICATIONS FOR CELL INVASION AND MIGRATION

1. **Assessment of ovarian cancer spheroid attachment and invasion of mesothelial cells in real time.**
   Bilandzic M, Stenvers KL.

2. **A real time chemotaxis assay unveils unique migratory profiles amongst different primary murine macrophages.**

3. **c-Myb regulates matrix metalloproteinases 1/9, and cathepsin D: implications for matrix-dependent breast cancer cell invasion and metastasis.**

4. **MicroRNA-200c Represses Migration and Invasion of Breast Cancer Cells by Targeting Actin-Regulatory Proteins FHOD1 and PPM1Ferences.**

5. **Comparative Analysis of Dynamic Cell Viability, Migration and Invasion Assessments by Novel Real-Time Technology and Classic Endpoint Assays.**

**KEY BENEFITS**

- Quantitative monitoring of cell migration or invasion in real time for up to hundreds hours.
- Label-free assay requires no fixation, staining or any other sample processing, dramatically reducing hands-on time.
- Easy quantification of the kinetics of migration or invasion.
- Rapid optimization of cell density and extracellular matrix density conditions.
- Non-invasive nature allows for quantitation of migrated/invaded cells by standard assays upon completion, typically revealing excellent correlation between Cell Index and migrated/invaded cell numbers.
Monitoring of T47D cellular response to H295R estrogen signaling. Estrogen and progesterone receptor positive T47D breast cancer cells were grown on xCELLigence E-plates. After a coculture insert containing H295R adrenal corticocarcinoma cells, known to produce estrogen and progesterone, was placed on the E-plate. The response of the T47D cells to the secreted hormones was suppressed by the application of estrogen synthesis inhibitors (A) Ketoconazole and (B) Anastrazole to the stimulatory H295R cells. (Data and figure adapted from Abassi YA., et. al. (2012).)
SELECT PUBLICATIONS FOR CELL-CELL INTERACTIONS

1. Dynamic assessment of cell viability, proliferation and migration using real time cell analyzer system (RTCA).
   Roshan Moniri M, Young A, Reinheimer K, Rayat J, Dai LJ, Warnock GL.
   Cytotechnology. 2014 Jan 19. [Epub ahead of print]

2. Using real-time impedance based assays to monitor the effects of fibroblast derived media on the adhesion, proliferation, migration and invasion of colon cancer cells.
   Dowling CM, Herranz Ors C, Kiely PA.
   Biosci Rep. 2014 Jun 17. [Epub ahead of print]

KEY BENEFITS

- Label-free environment allows detection of responses due to stimulation of endogenous receptors.
- Kinetic response profiles may be diagnostic for specific pathways.
- Real time data provides comprehensive information on cell responses over long time periods.
- E-Plate Insert offers an easy to setup co-culture platform with minimal handling.
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